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A Journal of Pulmonary Diseases

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MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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THE AMERICAN REVIEW OF TUBERCULOSIS

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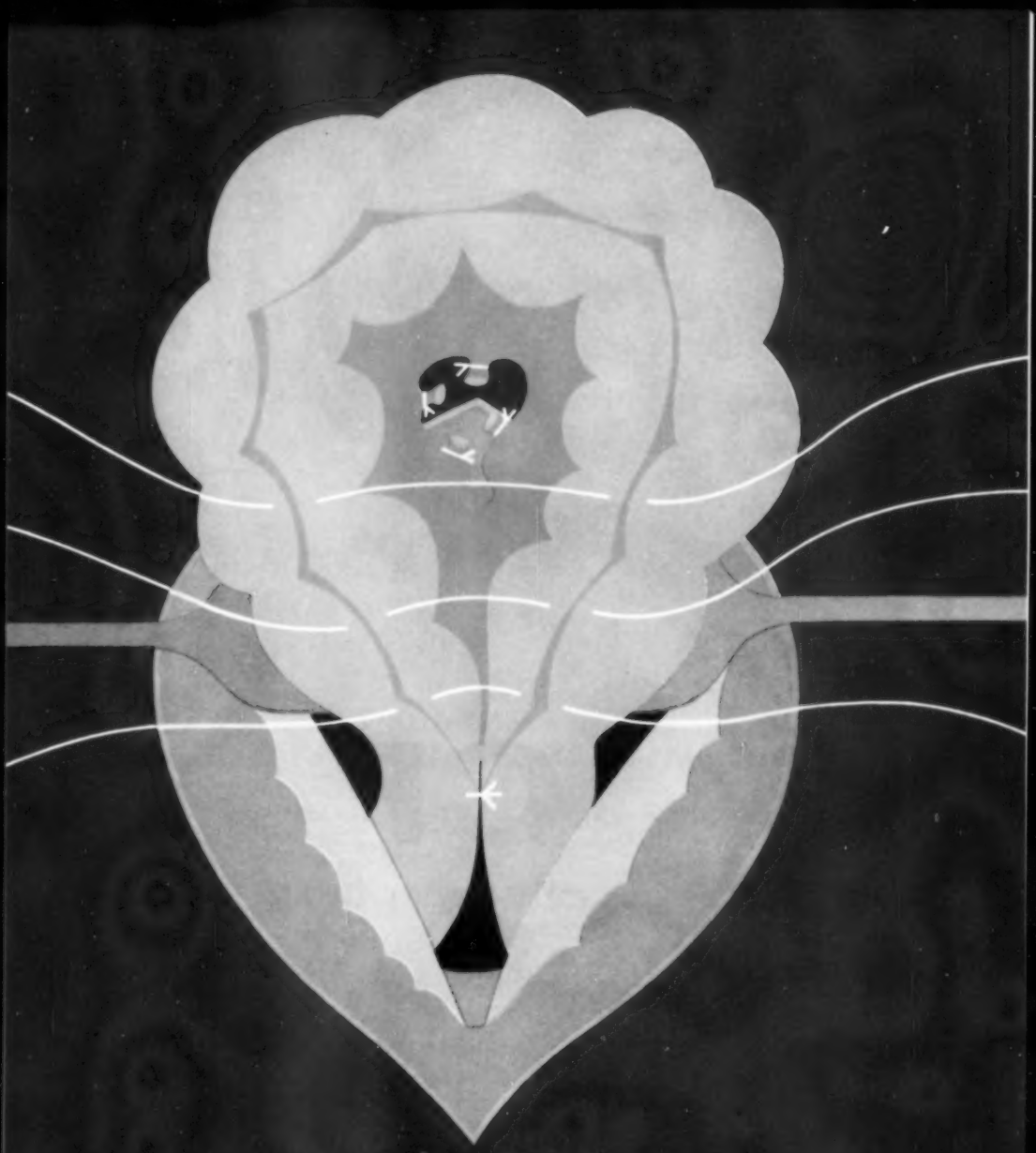

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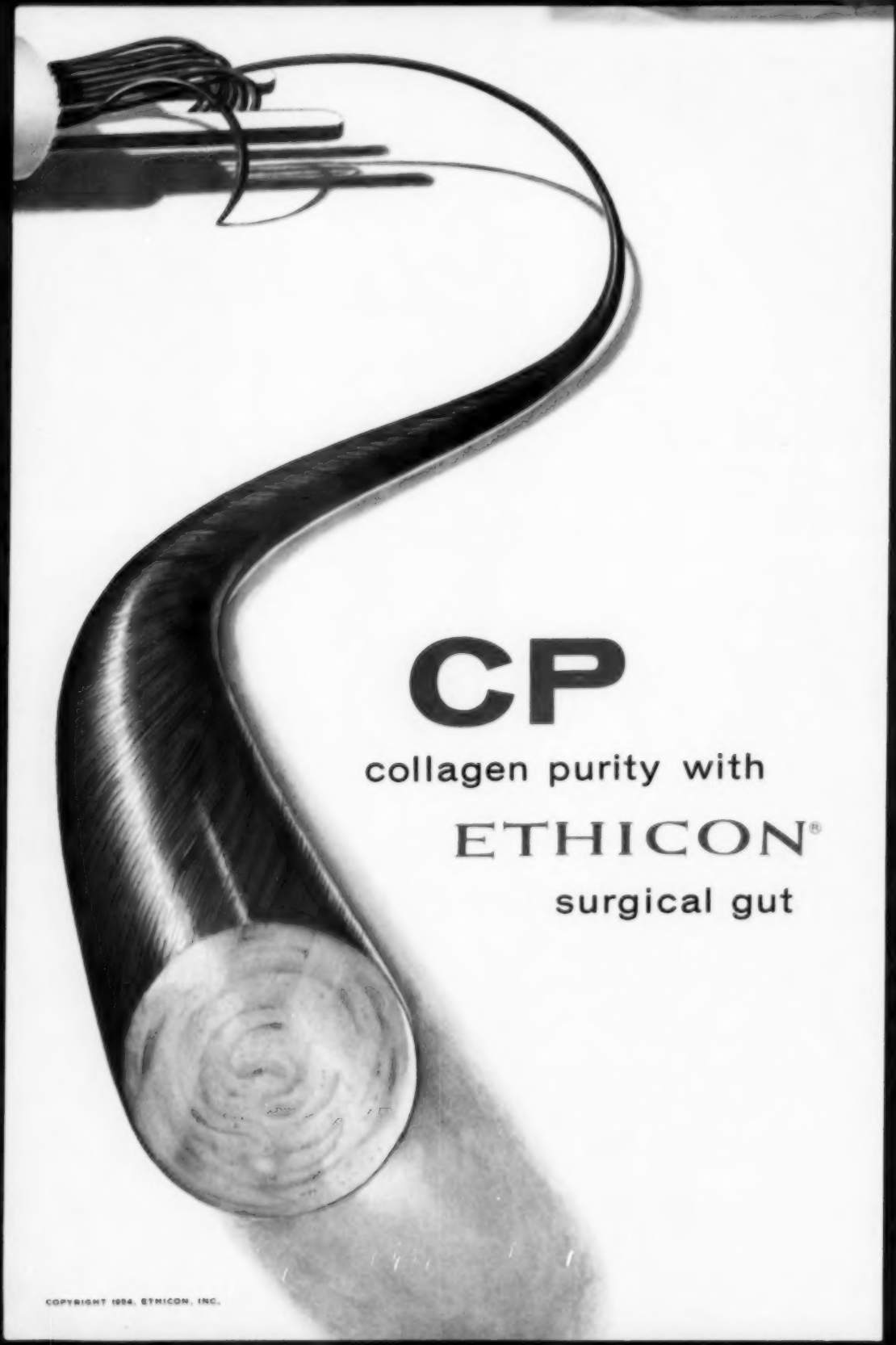

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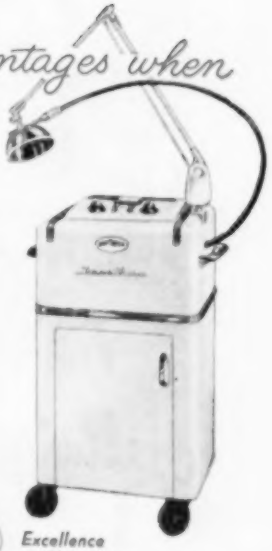

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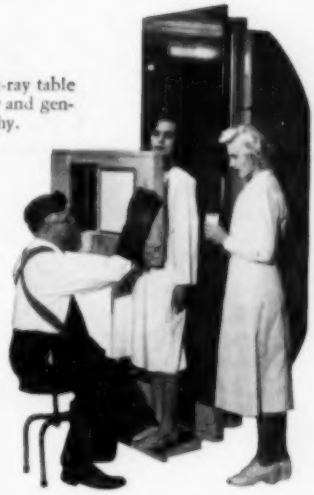

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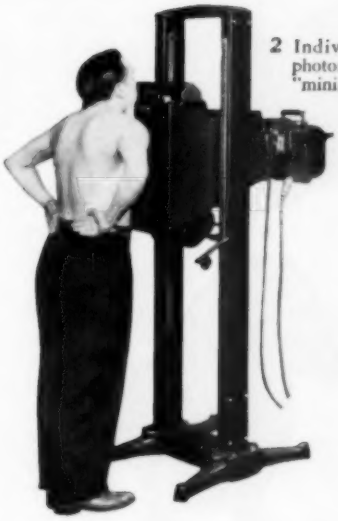

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ERRATA

In Volume 70, No. 4, 1954, "The Effects of Isoniazid on Patients with Multiple Sclerosis" by John F. Kurtzke and Louis Berlin, the fourth and fifth paragraphs of the summary should be changed to read as follows:

Of the comparison group admitted with mild to moderate disabilities (categories 1 through 5), a total of 117 patients, *53 per cent* were unchanged at the time of discharge, *34 per cent* were improved by one or more categories, and *13 per cent* were worse. In the group treated with isoniazid, 11 were in the mild to moderate categories on admission (categories 1 through 5), but on discharge 100 per cent of these had improved.

In the categories of moderate to severe impairment (categories 6 through 10), there were 58 patients in the comparison group. Of this group, *45 per cent* were unchanged, *24 per cent* were worse, and *31 per cent* had improved during hospitalization. In the group treated with isoniazid, there were 19 patients in categories 6 through 10. Of these, *85 per cent* improved, *10 per cent* were unchanged, and *5 per cent* (one patient) died while in the hospital.

In Volume 70, No. 3, 1954, "The Chemotherapy of Pulmonary Tuberculosis With Pyrazinamide Used Alone and in Combination with Streptomycin, Para-Aminosalicylic Acid, or Isoniazid" by William S. Schwartz and Ralph E. Moyer, the next to the last paragraph on page 415 should be changed to read as follows:

The concentrations of pyrazinamide in the blood were determined in 2 patients who were receiving 2.8 gm. daily. On the fifteenth day of treatment, the pyrazinamide concentrations were *57 and 46*γ per ml. at 8 a.m., *92 and 85*γ at 10 a.m., and *103 and 96*γ per ml. at 10 p.m., respectively.

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CANCER OF THE LUNG[1,2]

A Review of Experiences with 1,457 Cases of Bronchogenic Carcinoma

ALTON OCHSNER, C. JACK RAY, AND PAGE W. ACREE

(Received for publication May 17, 1954)

INTRODUCTION

With the development of more efficient methods for the treatment of pulmonary tuberculosis and with a concomitant increase in the incidence of malignant lesions of the bronchus, a physician interested in chest diseases must necessarily recognize the increasing importance of bronchogenic cancer. Although there are some who maintain that cancer of the bronchus is not increasing but that the apparent increase is due only to recognition of the condition at the present time which previously was misdiagnosed, this contention does not seem valid for two reasons. First, in the older age group, tuberculosis, which is the condition with which bronchogenic cancer is likely to be confused, has not decreased, whereas bronchogenic cancer is increasing more than any other cancer. Second, in those countries, particularly in Europe, where autopsies are done almost routinely, bronchogenic cancer has increased as it has in the United States; and it is doubtful that a competent pathologist is likely to overlook a bronchogenic cancer at autopsy. In Holland (1), for instance, cancer of the lung increased twenty four-fold in men and tenfold in women in the period from 1924 to 1951. In the United States, between 1914 and 1950, bronchogenic cancer increased in women from 0.6 per 100,000 population to 4.3 per 100,000 population, and in men the increase was from 0.7 to 19 per 100,000 population.

Cancer of the lung is increasing more than any other cancer in the body. In 1920, it represented 1.1 per cent of all cancers in the United States; in 1930, 2.2 per cent, and in 1948, 8.3 per cent. The writers have had the temerity to predict that in 1970, approximately twenty years hence, it will represent 18 per cent of all cancers, or approximately one in every five. As men are more

[1] From the Department of Surgery, School of Medicine, Tulane University, New Orleans, Louisiana; and the Ochsner Clinic.

[2] Presented before the Medical Session, as part of the session on *Nontuberculous Pulmonary Disease*, at the annual meeting of the National Tuberculosis Association, Atlantic City, New Jersey, May 17, 1954.

frequently involved than women, it is the belief of the writers that, in 1970, one of every two or three men with cancer will have cancer of the lung (2).

Although all cancers are increasing because cancer is primarily a disease of advancing age, and we are becoming a nation of older people, cancer of the lung follows a different pattern from all other cancers. All cancers except cancer of the lung increase with advancing age. A greater percentage of people eighty years of age will develop cancer than those seventy, and a greater percentage of those seventy will develop cancer than those sixty, and so on. However, cancer of the lung does not follow this pattern. There is a rapid increase in the incidence of cancer of the lung, much greater than that seen in any other cancer, to reach a peak age at the present time of approximately fifty-five years, following which there is a decrease in incidence of the disease.

This variation in the behavior of cancer of the lung is not new, but there has been an alteration in the curve. Fifteen years ago the peak incidence occurred at a later age and the peak was not as high; in other words, the peak incidence of cancer of the lung is becoming higher and is being reached at an earlier age than previously.

The reason for the differences in behavior and incidence of cancer of the lung from those of other cancer is, in the opinion of the writers, that persons who have smoked heavily for many years have subjected their hearts and blood vessels to the deleterious effects of tobacco. As a consequence, they are likely to develop coronary thrombosis and die before the carcinogenic effect of tobacco has a chance to exert itself. It might be stated that a possibly dubious advantage of cigarette smoking is that one might be prevented from having a cancer of the lung if he develops coronary disease and dies before he has a chance to develop lung cancer.

Although there are many who will disagree with the contention that cancer of the lung is caused by cigarette smoking, it is the writers' conviction that the unprecedented increase in the incidence of cancer of the lung is due almost entirely to cigarette smoking. There is a distinct parallel between the consumption of cigarettes and the increased incidence of cancer of the lung. Although this might be considered coincidental and is so considered by many, the fact that it is progressively increasing hardly supports this contention. In addition, experimental investigations have shown that cigarette smoke contains a carcinogen.

Many investigations have demonstrated the presence of carcinogens in tobacco, but in none except in the investigation performed by Wynder and Graham (3) was the experiment done in such a way that the cigarette smoking by humans is simulated. In this particular experiment a robot machine smoked twenty-four cigarettes at a time in a way comparable to that in which a human being smokes; that is, every sixty seconds a drag of two seconds is taken. The smoke obtained from the cigarettes was cooled, and the tarred residue was applied to the skin of animals three times a week. At the end of eight months, one benign tumor developed at the site of application. At the end of one year, one animal developed a cancer. At the end of two years, however, 44 per cent of the animals developed cancer at the site of the application of the tarred resi-

due and the cancer was similar to that seen in human beings in that the tumor metastasized, killed the animal, and was also transplantable.

The criticism is frequently made that one cannot compare animal cancer with human cancer; no attempt is being made to do so. However, it is well known that cancer of the lung can be produced by carcinogenic agents. The experiences in Schneeberg mines (4 *a*, *b*) demonstrate this without any question of a doubt. It is also a fact that cancer of the lung is increasing in an unprecedented manner more than any other cancer and that there is a distinct parallel between the consumption of cigarettes and the increase in the incidence of cancer of the lung. The investigations of Wynder and Graham demonstrate without any question that a carcinogen is contained in cigarette smoke. It is, therefore, a logical conclusion that the unprecedented increase in the incidence of cancer of the lung is due to cigarette smoking. The writers are so convinced of the causal relationship between cigarette smoking and cancer of the lung that they fear that, unless something is done to remove the carcinogenic factor from the tobacco in cigarettes, the incidence of cancer of the lung will increase in even more alarming proportions.

An argument which is frequently advanced against the causal relationship between smoking and cancer of the lung is that, in contradistinction to other areas in which epidermoid carcinomas are caused by carcinogens, premalignant lesions do not occur in the bronchial tree. Apparently, the fact that premalignant lesions are not found in the bronchi is due to the fact that pathologists seldom look for them. In a recent unpublished study made by Millard Costilow (5) (a senior medical student at Tulane University) on persons who died from other causes, it was found that in heavy smokers there were definite metaplastic changes which were probably precancerous and that there was considerable alteration from that seen in persons in the same age group who had not smoked (figures 1 and 2).

Another argument which is advanced is that, if there is causal relationship between cigarette smoking and cancer of the lung, there should be a commensurate increase in the death rate from cancer of the larynx, which there is not. This difference between the fatality incidence of cancer of the lung and that of cancer of the larynx is undoubtedly due to the fact that cancer of the larynx is readily diagnosed and is readily amenable to therapy. Cancer of the larynx *is* increasing as much as cancer of the lung, but the fatality incidence is lower in cancer of the larynx because of the high curability rate.

Although both whites and nonwhites have cancer of the lung, it is found more frequently among white people. In the Charity Hospital in New Orleans, an ideal opportunity has been afforded to study racial incidence because both the white and nonwhite races are admitted with about equal frequency. In that institution, cancer of the lung is found in white people in 177 per 100,000 admissions as compared with 130 in the nonwhites. Men are more frequently involved than women. In the New Orleans series of 1,457 cases, 90 per cent were in men and 10 per cent in women (figure 3). The writers are of the opinion that, in spite of the fact that women are beginning to smoke almost as much as men, cancer of the lung will never become as frequent in women because, in all prob-

FIG. 1. Normal pseudostratified columnar ciliated epithelium from bronchus of non-smoker. (H & E × 120) (*Courtesy of Dr. Millard Costilow*).

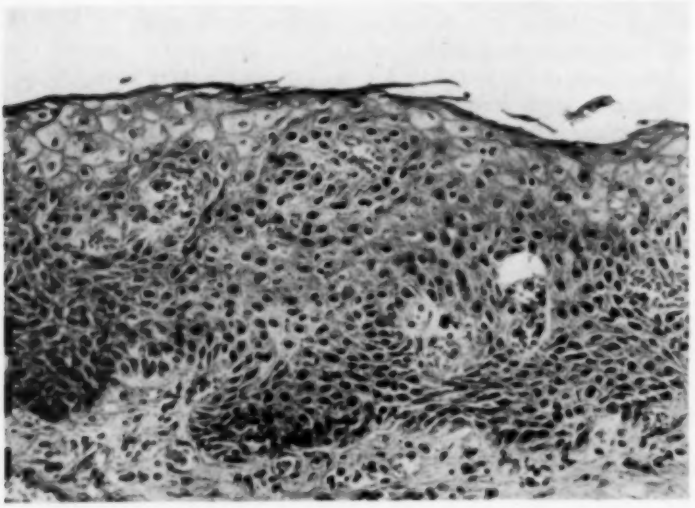

FIG. 2. Marked squamous metaplasia of bronchial epithelium seen in excessive smoker. There are marked nuclear changes, blood vessel penetration into the epithelium, hyperchromatism, and actual keratinization of the surface layers. (H & E × 120) (*Courtesy of Dr. Millard Costilow*).

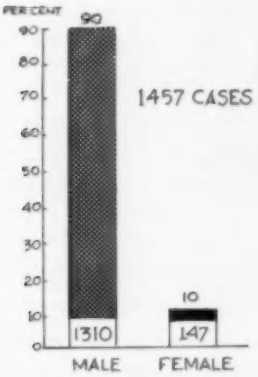

FIG. 3. Sex incidence in 1,457 cases of bronchogenic carcinoma.

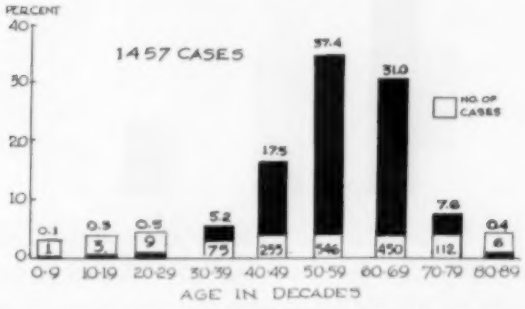

FIG. 4. Age incidence in 1,457 cases of bronchogenic carcinoma

ability, men are more susceptible to this condition than women. Evarts Graham, (6), on the other hand, is of the opinion that it will become as common in women when they smoke as much as men do.

As in most cancers, cancer of the bronchus is primarily a disease of advancing age. The greatest incidence in the present series was in the sixth decade, 37.4 per cent, followed by a frequency in the seventh decade of 31 per cent and in the fifth decade of 17.5 per cent (figure 4). Of the resected cases, 39.6 per cent were in the sixth decade, 31.9 per cent in the seventh decade, and 17.2 per cent in the fifth decade. Thus, almost 90 per cent of the cases of bronchogenic cancer in the writers' series occurred in patients between fifty and eighty years of age.

PATHOLOGY

Location: In this series, the right lung was involved somewhat more frequently than the left, i.e., in 54 per cent of the cases, compared with left-sided involve-

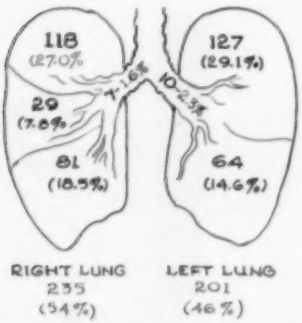

FIG. 5. Anatomic location of lesion

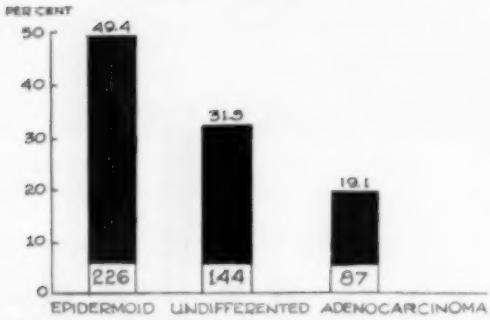

FIG. 6. Histologic type of 457 cases of bronchogenic carcinoma

ment in 46 per cent (figure 5). Of great importance is the fact that the upper lobes are involved more frequently than other portions. In the present series, the right upper lobe was involved in 27 per cent and the left upper lobe in 29 per cent of the cases. Thus, in more than 55 per cent of the cases, the upper lobes were involved. This is of practical importance because these tumors are frequently in a position which is difficult to visualize bronchoscopically. It is for this reason that the bronchoscopic visualization is relatively infrequent.

Nature of tumor: The most frequent type is an epidermoid tumor, and the writers believe that this is the type of tumor which is produced by a carcinogen. In the present series, 49.4 per cent of the tumors were epidermoid carcinoma, 31.5 per cent were undifferentiated, and 19.1 per cent were adenocarcinoma (figure 6). In the males, epidermoid carcinomas were also the most frequent, representing 53 per cent; with undifferentiated carcinomas representing 32 per cent, and adenocarcinomas, 15 per cent, of the total (figure 7). On the other hand, in women, 21 per cent of the tumors were epidermoid, 28 per cent were undifferentiated, and 51 per cent were adenocarcinomas. Of the epidermoid carcinomas, 95 per cent occurred in men and 5 per cent in women. Of the undifferentiated tumors, 89 per cent occurred in men and 11 per cent in women. Of the adenocarcinomas, 69 per cent were in men and 31 per cent in women.

FIG. 7. Sex incidence in histologic type of 457 resected cases of bronchogenic carcinoma

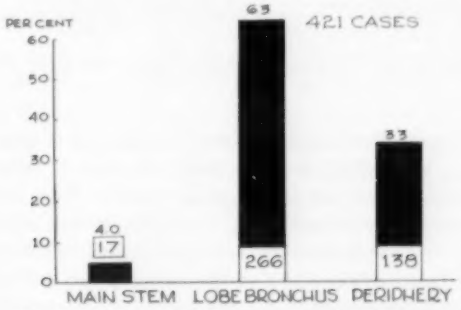

FIG. 8. Location of lesion in bronchopulmonary tree in 421 cases of bronchogenic carcinoma.

The location of the bronchogenic carcinoma is usually the lobar bronchus. In 4 per cent of the cases in the present series, the main stem bronchus was involved; in 63 per cent, the lobar bronchus was involved; and in 33 per cent, a peripheral bronchial ramification was involved (figure 8). As is commonly known, more adenocarcinomas than other types of tumor occur in the periphery. Of all of the cases of adenocarcinoma, 4 per cent were in the main stem bronchus, 55 per cent in the lobar bronchi, and 28 per cent had arisen in the periphery. These percentages for the undifferentiated carcinomas were 4, 59, and 37, respectively (figure 9).

CLINICAL MANIFESTATIONS

There are no classical manifestations of bronchogenic cancer. In fact, cancer of the bronchus probably does not cause any symptoms at all until it has attained considerable size except in the fortunate person who has an extremely small lesion in which early ulceration occurs. In such an instance, hemoptysis may be an early manifestation. Rarely, a bronchogenic cancer may produce no symptoms referable to the lung, and the first manifestation may be a distant metastasis. However, this represents the exception. There are undoubtedly many cases of bronchogenic cancer which are present for relatively long periods of time before symptoms are produced.

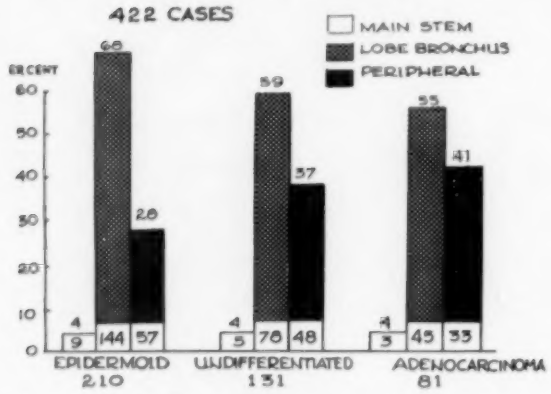

FIG. 9. Location of lesion in bronchopulmonary tree in 422 cases of bronchogenic carcinoma.

Respiratory symptoms: Frequently the early symptoms of bronchogenic cancer are those of a lower respiratory tract infection. In fact, in most of the patients a diagnosis of influenza or viral pneumonitis is made. This is certainly understandable because a lesion occluding the bronchus favors infection of the lung parenchyma distal to the obstruction, and the clinical manifestations are those of a pneumonitis. In more than half of the cases in the writers' series, a diagnosis of a pneumonitis had been made.

Undoubtedly the most frequent manifestation of bronchogenic cancer is a cough, but, because all excessive cigarette smokers have a persistent cough, it is likely to be disregarded. If, however, there is a change in the character of the cough, it is of significance. Hemoptysis always demands investigation because it means an ulceration of the respiratory tract. In all such persons, particularly men more than forty years old who have been heavy smokers, it is obligatory either to confirm or to exclude the diagnosis of bronchogenic cancer. Another frequently early manifestation is thoracic discomfort, which also demands investigation. Wheezing may be the earliest manifestation of bronchogenic cancer because it is an indication of bronchial obstruction. It is of much less significance in a person who has been asthmatic all his life, but, in a man more than forty years old who has been a heavy smoker and previously had had no wheezing, it is indicative of bronchogenic cancer until proved otherwise. Late manifestations of bronchogenic cancer are fever, pain, digestive disturbances, and emaciation, which usually are indicative of extensive disease. In the resected cases of the present series, cough had been present in 87 per cent, weight loss in 66 per cent, chest pain in 61 per cent, hemoptysis in 52 per cent, previous respiratory tract infection in 52 per cent, dyspnea in 47 per cent, weakness in 46 per cent, wheezing in 12 per cent, night sweats in 11 per cent, and voice change in 6 per cent (figure 10).

Involvement of phrenic or recurrent laryngeal nerves: Although there are many

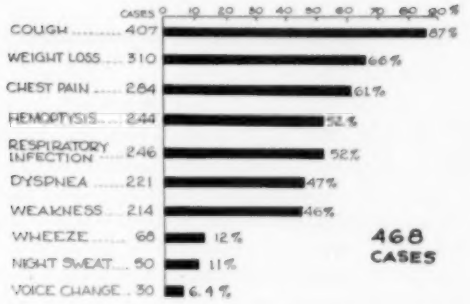
CASES 0 10 20 30 40 50 60 70 80 90 %
COUGH 407 87%
WEIGHT LOSS 310 66%
CHEST PAIN 284 61%
HEMOPTYSIS 244 52%
RESPIRATORY INFECTION 246 52%
DYSPNEA 221 47%
WEAKNESS 214 46%
WHEEZE 68 12%
NIGHT SWEAT 50 11%
VOICE CHANGE 30 6.4%
468 CASES

FIG. 10. Symptoms in cancer of the lung in resected cases

thoracic surgeons who accept evidence of involvement of the recurrent laryngeal or phrenic nerves as an indication of inoperability, the writers have not been willing to do so. Whereas involvement of either one or both of these nerves indicates extension of the lesion beyond the lung to involve the nerve, it does not necessarily mean that the lesion cannot be resected or that the patient cannot be cured. Unless there is other evidence of inoperability, it is believed that the patient should be given advantage of a thoracic exploration. In the present series, there is one patient who had involvement of the recurrent laryngeal nerve by a tumor and who is alive and apparently well seven years after the pneumonectomy. Had recurrent laryngeal nerve paralysis been an indication of inoperability, this patient would undoubtedly have succumbed by now.

DIAGNOSIS

Roentgenography: The diagnosis of cancer of the lung is not difficult if one only considers the possibility of its existence. Roentgenography is particularly valuable because of the ease with which a chest roentgenogram can be obtained. It is hoped that earlier diagnoses of bronchogenic cancer will be made in the future by routine roentgenography of all men more than forty years old who have smoked excessively for a number of years.

A lesion which is large enough to cast a shadow can be detected on the roentgenogram, and investigations must be instituted to verify or exclude the diagnosis of bronchogenic cancer. Difficulty is encountered in hilar lesions because in this area small lesions can be detected only with difficulty. Whether the use of supervoltage techniques for visualization of these hilar lesions will become practicable, only time will tell. The original investigations by Pendergrass and his associates (7) indicate that these will offer a possible method of detecting small hilar lesions.

In some of the early lesions which are so small as to occlude the bronchus incompletely, obstructive emphysema may be present. This is due to the fact that the bronchus is not a rigid tube but dilates during inspiration and contracts during expiration. As a result of dilatation during inspiration, air gets beyond the tumor into the lung distally but, during expiration, as a result of the con-

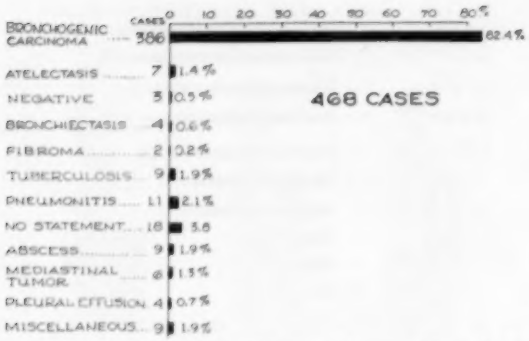

FIG. 11. Roentgenographic diagnosis in 468 resected cases of bronchogenic carcinoma

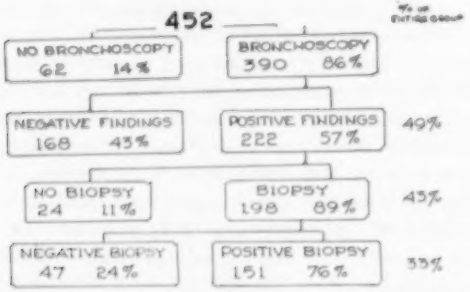

FIG. 12. Bronchoscopic results in 452 resected cases of bronchogenic carcinoma

traction of the bronchus on the tumor, air cannot get out and is trapped, producing the obstructive emphysema. If the tumor completely occludes the bronchus, atelectasis with a tell-tale shifting of the mediastinum toward the affected side, development of the characteristic shadow, and visible elevation of the diaphragm occur. Occasionally, bronchography is of value in detecting peripherally located lesions or those in the upper lobe. A correct diagnosis was made in 82 per cent of the cases in the present series by the roentgenographic findings (figure 11).

Bronchoscopy: Bronchoscopy is indicated in most cases of bronchogenic cancer except in a small peripheral lesion in which bronchoscopy would probably give little information. It is unfortunate that by bronchoscopy only a relatively small number of tumors can be visualized. In only 33 per cent of the present cases was it possible to make a diagnosis on bronchoscopy (figure 12). This is due to the fact that the majority of the tumors are in the upper lobes and some are in the periphery beyond bronchoscopic vision. Bronchoscopy is of value, however, not only in making it possible to visualize those tumors within bronchoscopic vision, but also because it will often give considerable information concerning operability. In those instances in which there is fixation of the main stem bronchi, the case is probably inoperable.

Cytologic examination of sputum or bronchial washings: Cytologic examination of the bronchial secretion is, in the writers' experience, the most valuable method of making a positive diagnosis of bronchogenic carcinoma. Material can be obtained either by bronchial aspiration or by expectoration. Whereas, in the present series, higher instance of positive diagnoses, specimen for specimen, has been made from study of bronchial aspiration material, a greater number of *cases* have been diagnosed positively from examination of the sputum. This is due simply to the fact that, whereas only one bronchial aspiration is obtained, many sputum specimens can be examined. In 68 per cent of the cases, it was possible to make a positive diagnosis of bronchogenic cancer by cytologic examination.

Aspiration biopsy: Aspiration biopsy has been advocated by many. Although it is a valuable method of making a diagnosis of peripherally located lesions, it is believed that it should not be used in the patient in whom a curative procedure might be possible because of the danger of implantation of tumor cells along the course of the aspirating needle. Instead, its use should be limited to the patient with an obviously inoperable lesion in whom a positive microscopic diagnosis is advisable.

Surgical exploration: In 80 per cent of the cases of the present writers, it has been possible to make a positive preoperative diagnosis; in 20 per cent, however, it was not possible to make a positive diagnosis before thoracic exploration. It is believed, therefore, that thoracic exploration is a justifiable method of diagnosis and definitely should be used in any patient in whom a bronchogenic cancer might exist unless there is an obvious contraindication to the procedure. At the time the chest is opened, several procedures can be undertaken to determine whether bronchogenic cancer is present or not. If the lesion is in the periphery, it can be removed by wedge resection, or a simple biopsy can be done. In other instances, segmental resection or lobectomy is performed. An immediate frozen section is made, and if the lesion proves to be carcinoma, a radical pneumonectomy with an en bloc excision of all the mediastinal nodes should be done. If, however, there is any question about the lesion being benign or malignant, it is better to discontinue the operation and wait until the permanent section is examined, even at the risk of having to perform a pneumonectomy later if the permanent section proves that the lesion is malignant.

TREATMENT

The only curative treatment of bronchogenic carcinoma is surgical extirpation. Although there are some who are of the opinion that lobectomy can and should be used in the treatment of bronchogenic cancer, the writers are definitely of the opinion that lobectomy is not a good cancer operation. There is no question but that some patients with bronchogenic cancer can be cured by the removal of a lobe, but it is strongly believed that many more patients would be cured if a radical operation, consisting of pneumonectomy with an en bloc excision of all the mediastinal nodes, were done. Indeed, it is believed that the only indication for lobectomy for bronchogenic cancer is preoperative evidence obtained from careful pulmonary function studies that a pneumonectomy could not be tolerated.

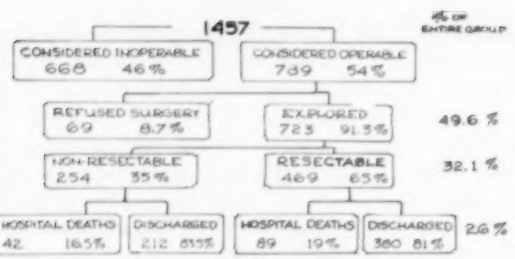

FIG. 13. Results in 1,457 cases of bronchogenic carcinoma

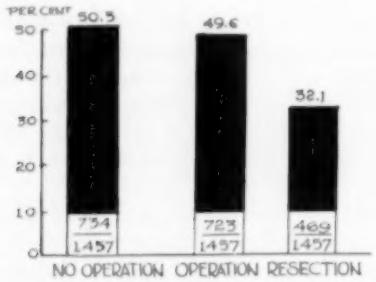

FIG. 14. Management of 1,457 cases of bronchogenic carcinoma

Operable versus inoperable cases: Unfortunately, many cancers of the lung are far advanced by the time a diagnosis is made. In the present series of 1,457 cases, 668 (46 per cent) were hopelessly inoperable when first seen (figure 13). Of the 789 which were considered to be operable, 69 (8.7 per cent) of the patients refused surgery and 723 had exploratory operations. Two hundred fifty-four (35 per cent) of the 723 patients were found to have non-resectable lesions. Of this group, 42 (16.5 per cent) died in the hospital. Of 469 patients in whom a resection was accomplished, 89 (19 per cent) died in the hospital; 380 (81 per cent) were discharged from the hospital. The management of the 1,457 patients may be summarized as follows: No operation was done in 50.3 per cent, 49.6 per cent were operated upon, and, in 32.1 per cent, resections were performed (figure 14).

Palliative resection: In 73 per cent of the patients, the resection was a palliative one, a procedure chosen arbitrarily when there is extension beyond the lung (figure 15). For some reason or other, thoracic surgeons generally have been reluctant to perform palliative resections in the treatment of bronchogenic cancer. It has been difficult for the present writers to understand this philosophy. There are few, if any, abdominal surgeons who would hesitate to resect a malignant lesion of the colon in a patient with a metastatic nodule of the liver. Even though the patient will die of his cancer as a result of metastasis, he will certainly be more comfortable and will live longer because of relief of obstruction. It is believed that one is equally justified in performing palliative resection for bronchogenic cancer in cases in which there is extension beyond the lung. Even though

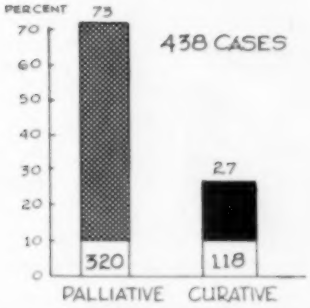

FIG. 15. Supplementary operative procedures in 438 cases of bronchogenic carcinoma

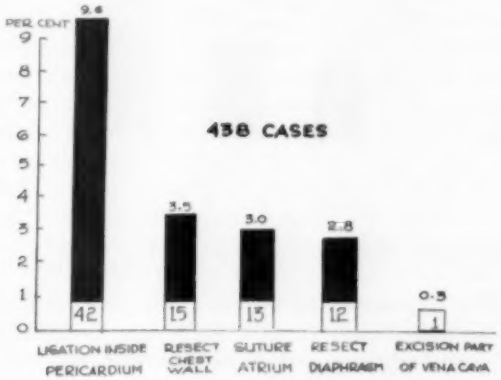

FIG. 16. Incidence of type of resection

the patient may not survive any longer than if he were not operated upon, he will be relieved of symptoms produced by complications of the tumor, e.g., suppuration or hemorrhage.

Advanced lesions: The writers have operated upon many patients with advanced lesions (figure 16). In 9.6 per cent, ligation of the pulmonary vessels within the pericardium was obligatory; in 3.5 per cent, a resection of the major portion of the chest wall was performed; in 3 per cent the atrium was sutured; in 2.8 per cent, a major portion of the diaphragm was removed; and in one case, partial excision of the vena cava was done.

Causes of death: The causes of death varied in the 84 fatal cases. The greatest causes of death were cardiovascular complications, 43.8 per cent (figure 17). These complications probably never can be completely eliminated because it must be recalled that the majority of patients are in the sixth and seventh decades of life. Thirty-three and seven-tenths per cent were due to respiratory complications, 15.7 per cent to hemorrhage, and 6.7 per cent were due to miscellaneous causes. The mortality rate in this series is high because of the fact that advanced

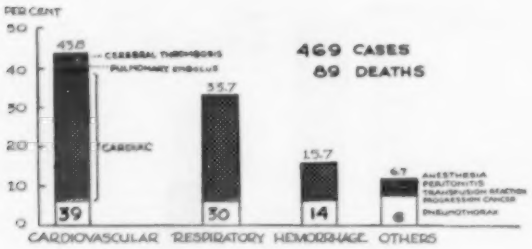

FIG. 17. Causes of hospital deaths

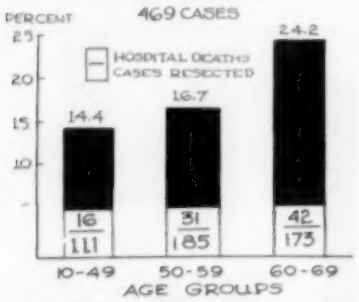

FIG. 18. Mortality rate according to age of patient

lesions were present in almost three-fourths of the patients. The mortality rate, as is obvious, is dependent upon the age of the patient. Of those patients less than fifty years of age, 14.4 per cent died; of those between fifty and fifty-nine, 16.7 per cent died; and of those from sixty to eighty years, 24.2 died (figure 18).

Delay before diagnosis and surgery: It is lamentable that only 49 per cent of the cases were operable and only 32 per cent were resectable at the time the patients were first seen. This is understandable, however, when it is realized that there was an average delay of 8.7 months from the onset of the first symptom until therapy was instituted. A delay of more than 6 months in any malignant process is hazardous. It is of interest that delay was longer in the group of patients in whom a resection was possible than in the group in whom a resection could not be done, 10.5 months and 7.5 months, respectively. Although this seems paradoxical, it simply indicates that a slowly growing tumor is likely to produce relatively few manifestations, and a diagnosis is not likely to be suspected until after a long delay. On the other hand, a rapidly growing tumor, which produces early and marked symptoms, is suspected of being malignant at an earlier time but, unfortunately, not early enough to permit resection. This does not mean, however, that, had the diagnosis been made within six weeks to two months after the onset of symptoms, the lesion would not have been resectable.

It is also disturbing that there was a longer delay in the private cases than in the ward cases, 9.3 months and 7.5 months, respectively. In determining who

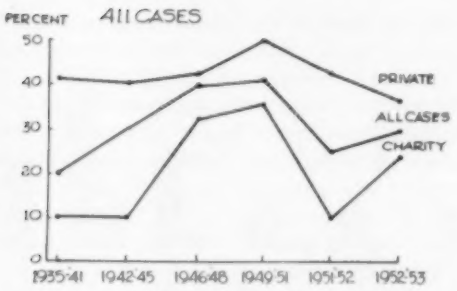

FIG. 19. Incidence of resection by periods.

was responsible for the delay, it was found that the patient was responsible for a delay of 2.8 months and the physician for a delay of 5.5 months. In the ward cases, the patient was responsible for a delay of 3 months and the physician for a delay of 3.6 months; but, in the private cases, the patient was responsible for a delay of 2.6 months and the physician was responsible for a delay of 6.2 months.

Confusion with atypical pneumonia: It is to be deplored that there was this longer delay in the private cases than in the ward cases. It was probably due to the fact that the private patient, who was able to afford hospitalization and in whom a diagnosis of viral pneumonia was made, was often sent to a private institution where he was treated for weeks or months for an "atypical pneumonitis." He undoubtedly had some benefit because of control of the secondary infection by antimicrobial therapy, but the true condition (bronchogenic cancer) was possibly not considered until relatively late. It is unfortunate that the profession too frequently considers *atypical* pneumonitis as synonymous with *viral* pneumonitis. Approximately five years ago many clinical reports concerning viral pneumonitis were published and, because the pneumonic process was described as atypical, the profession has become conditioned to consider all atypical pneumonias as viral pneumonias. Probably because of this, the resectability incidence in lung cancer has decreased in recent years, which is unfortunate (figure 19). Although it is true that a man more than forty years old who has been a heavy smoker can have viral pneumonitis, the writers are equally convinced that his chances of having a bronchogenic cancer are even greater. It is imperative, therefore, that in all such patients in whom a prompt and complete subsidence of all clinical manifestations does not occur within a period of a few weeks, the possibility of an underlying bronchogenic neoplasm be considered and steps be taken either to exclude or to confirm such a diagnosis. A man more than fifty years of age who has been a heavy smoker for twenty years has fifty times the chance of developing bronchogenic cancer as a man of the same age who has never been a cigarette smoker.

RESULTS

In a consideration of any disease, particularly a malignant disease, the long time-survival rates are of greatest importance. Although much is to be desired in

the results in the surgical treatment of bronchogenic cancer, a great deal is being accomplished in spite of the fact that there was an average delay of 8.7 months from the onset of the first symptoms until definitive therapy was instituted. In 13.5 per cent of the resected cases, the patients are alive at the end of five years and are apparently free of the disease. In a consideration of the survival rates, it has been assumed that all persons who die following an operation, whether they die during the operation or any time afterward, die of the disease. Although this is an extremely critical way to evaluate results, because only 85 per cent of the general population at this age without bronchogenic carcinoma would be alive at the end of five years, the writers believe that it is only in this way that the personal equation can be eliminated. There are a number of patients in the series who have died after operation and in whom no evidence of residual carcinoma was found at autopsy. Nevertheless, they have all been considered to have died of cancer, and no exceptions have been made in the assumption that all patients who died during or after operation died of their neoplastic disease.

Of all of the patients in whom a resection was done for carcinoma, 47.5 per cent were alive at the end of six months, 33 per cent at the end of one year, 20 per cent at the end of two years, and 13.5 per cent at the end of five years (figure 20). The survival rates in those patients in whom the lesion had extended beyond the lung and in whom a palliative resection was done were as follows: 37 per cent were alive at the end of six months, 24 per cent at the end of a year, 14 per cent at the end of two years, and 6.6 per cent at the end of five years (figure 21). In those patients in whom the lesion was apparently limited to the lung, 74 per cent were alive at the end of six months, 56.5 per cent at the end of one year, 39 per cent at the end of two years, and 31 per cent at the end of five years (figure 21).

The writers are extremely proud of the results that have been obtained in the patients with palliative resection because it is believed that only by adopting the philosophy of doing resections in advanced cases has it been possible to obtain a 7 per cent salvage rate in these persons. If these patients had not been subjected to resection and had they been considered inoperable because of the extent of their disease, the writers are convinced that they would not have survived. This

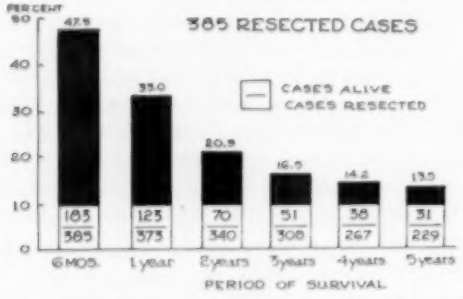

FIG. 20. Gross survival rate in resected cases. All patients who died were considered to have died of the disease.

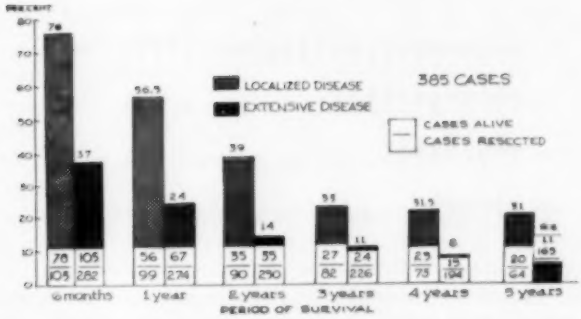

FIG. 21. Comparison of survival rates after resection according to extension and localization of lesion.

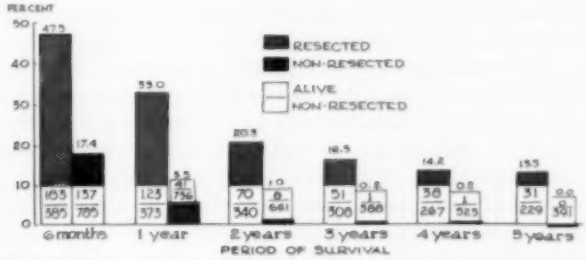

FIG. 22. Comparison of survival in resected and in non-resected cases

is substantiated by the relatively poor results obtained in those cases in which resection was not done, admitting that, with the exception of the group of patients who refused surgery, the nonresected cases represented more advanced disease than those in which resection was done. In all of the cases, however, in which a resection was not done, other palliative procedures such as nitrogen mustard administration, X-ray therapy, or a combination of both were used. Of this latter group, only 17.4 per cent were alive at the end of six months, 5.5 per cent at the end of a year, 1 per cent at the end of two years, and only one of 523 is alive at the end of four years (figure 22). It is thus seen that no other type of therapy except resection offers any approach to a cure.

It is of interest in the computation of the survival rates of the patients who have been subjected to resection, either palliative or curative, that there is a rapid decrease in the survival up to the second year, following which the decrease is much less. It may, therefore, be said that the person who has a resection of his lung for bronchogenic cancer, whether it is done as a palliative or curative procedure, and who is alive and apparently free from disease at the end of two years has a good chance of being well. This is true not only of bronchogenic carcinoma but is true of gastric cancer, colonic cancer, and uterine cancer.

To recapitulate the results, 85 per cent of the general population in this age

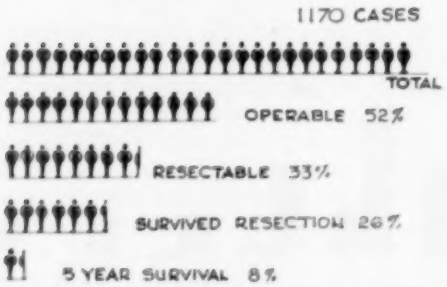

FIG. 23. Results obtained in entire series based upon present-day management

group are alive at the end of five years; 31 per cent of those with bronchogenic cancer in which the lesion was limited to the lung, 13.5 per cent of all those subjected to resection, and 6.6 per cent of those with extensive disease. Of the entire group, 8 per cent are alive at the end of five years.

The results observed in the present Tulane series of 1,457 cases indicate that much must be done in the treatment of bronchogenic cancer, since only 8 per cent of the entire group of patients are alive at the end of five years (figure 23). The fact that only 33 per cent of the entire group were resected shows that there is much to be accomplished, but that it can be accomplished only by earlier diagnosis and institution of curative therapy at a time when the lesion is still limited to the lung. In order to accomplish this, it is imperative that all persons, particularly men more than forty years old, who have unexplained thoracic discomfort, be considered to have bronchogenic cancer until proved otherwise. Moreover, in order to detect the asymptomatic lesions, it is imperative that all men more than forty who have been heavy cigarette smokers should have chest roentgenograms at least every six months, preferably every three months, so that, if and when they do develop bronchogenic cancer, it can be detected at a time when it probably is still limited to the lung and is curable.

SUMMARY

Cancer of the lung is increasing more than any other cancer in the body; and is due, the writers believe, to the carcinogenic effect of cigarette smoking. It is a disease primarily of men, principally in the sixth and seventh decades of life.

Approximately half of bronchogenic cancers are epidermoid; 31 per cent, undifferentiated; and 19 per cent, adenocarcinomas. The upper lobe bronchi are involved more frequently than any other bronchi. Any man past forty years of age who has been a heavy smoker should be suspected of having bronchogenic cancer if he has an unexplained thoracic discomfort. Viral pneumonitis should be viewed with suspicion in all such persons because of the likelihood that an associated infection in bronchogenic cancer can simulate viral pneumonitis.

The diagnosis of bronchogenic cancer is usually not difficult if the physician will only think of the possibility of its existence. All men past the age of forty

who have been heavy smokers should have a routine chest roentgenogram at least every six months. All shadows should be considered suspicious of carcinoma. Unfortunately, bronchoscopy makes a positive diagnosis in only approximately one-third of all cases. By cytologic examination of the sputum, a correct diagnosis can be made in approximately 68 per cent of cases. In 20 per cent of instances, it is necessary to resort to thoracic exploration in order to make a diagnosis.

In a series of 1,457 cases, 789 were considered operable, 723 were explored, of which a resection was possible in 469. There was an operative mortality rate of 19 per cent. Palliative resection was done in 73 per cent of the cases.

The fact that only 32 per cent were resectable at the time they were first seen was undoubtedly due to the fact that there was a long delay, an average of 8.7 months, from the onset of the first symptoms until therapy was instituted.

Thirteen and five-tenths per cent of the patients who had resections are alive at the end of five years and are apparently free from the disease. Since only 85 per cent of the general population sixty to seventy years old without cancer of the lung are alive, the results are better than they appear. Of the patients who had extensive involvement and in whom palliative resection was done, 6.6 per cent were alive at the end of five years. In a group of patients in whom no resection was done, only 17 per cent survived six months and only 1 per cent were alive at the end of two years even though other palliative therapy such as nitrogen mustard or irradiation was used. It is obvious that, in order to increase the salvage rate in bronchogenic cancer, the diagnosis must be made earlier, and this can be accomplished only by having men who have been heavy smokers have routine chest films at least every six months.

SUMARIO

Cáncer del Pulmón

El cáncer pulmonar va aumentando más que el de ninguna otra parte del cuerpo, debiéndose esto, según creen los AA., al efecto carcinógeno del fumado de cigarrillos. Es una enfermedad primariamente de los hombres, principalmente en el sexto y el séptimo decenios de la vida.

Aproximadamente la mitad de los cánceres broncógenos son epidermoideos; 31 por ciento no están diferenciados; y 19 por ciento son adenocarcinomas. Los bronchios de los lóbulos superiores se afectan más a menudo que ningún otro bronquio. Todo varón de más de cuarenta años que haya sido fumador inveterado debe considerarse como sospechoso de padecer de cáncer broncógeno si tiene un malestar torácico inexplicado. En todos esos individuos, debe considerarse con sospecha la neumonitis viral, dada la probabilidad de que, en el cáncer broncógeno, una infección concomitante simule neumonitis viral.

El diagnóstico de cáncer broncógeno no suele ser difícil si el médico solamente recuerda su posible existencia. Todo varón que haya cumplido cuarenta años y sido fumador obstinado debe hacerse tomar una radiografía torácica a los menos cada seis meses. Toda sombra observada debe considerarse como sospechosa de carcinoma. Por desgracia, la broncoscopia no hace un diagnóstico positivo más que en la tercera parte aproximadamente de los casos. Con el examen citológico del esputo, cabe hacer un diagnóstico acertado aproximadamente en 68 por ciento de los casos. En 20 por ciento de éstos, es necesario dirigirse a la exploración torácica para formular el diagnóstico.

En una serie de 1,457 casos, 789 fueron considerados como operables y se exploró a 723,

siendo posible la resección en 469 de los últimos. La mortalidad operatoria representó 19 por ciento. En 73 por ciento de los casos, se ejecutó una resección paliativa.

El hecho de que no fueran resecables más de 32 por ciento al observar a estos enfermos por primera vez procede sin duda de que hubo una gran tardanza, un promedio de 8.7 meses, desde la iniciación de los primeros síntomas hasta la institución de la terapéutica.

Un 13.5 por ciento de los enfermos en quienes se hicieron resecciones están vivos al cabo de cinco años y aparentemente exentos de la dolencia. Como a dicha edad no están vivos más que 85 por ciento de la población general que no tiene cáncer pulmonar, los resultados son mejores de lo que parecen. De los enfermos que ten an invasión extensa y en quienes se verificó una resección paliativa, 6.6 por ciento estaban vivos al cabo de cinco años. En un grupo de enfermos en el que no se hicieron resecciones, solamente 17 por ciento sobrevivieron seis meses y solamente 1 por ciento estaban vivos al cabo de dos años, a pesar de haberse usado otra terapéutica, como mostaza nitrogenada o irradiación. Resulta manifiesto que, a fin de aumentar el coeficiente de rescates en el cáncer broncógeno, hay que hacer el diagnóstico antes, lo cual sólo puede lograrse haciendo que los fumadores sempiternos se hagan tomar radiográfias torácicas de las corrientes a lo menos cada seis meses.

RESUME

Le cancer du pumon est en augmentation par rapport à tout autre cancer de l'organisme. Selon les auteurs, ceci est dû à l'action carcinogène de la fumée des cigarettes. C'est principalement une affection de l'homme, surtout aux environs de soixante à soixante-dix ans.

Approximativement la moitié des cancers bronchiogènes sont épidermoïdes; 31%, non différenciés; 19%, des adénocarcinomes. Les bronches du lobe supérieur sont plus fréquemment intéressées que les autres. Chez tout grand fumeur ayant plus de quarante ans et qui se plaint de troubles thoraciques, le cancer bronchigène doit être envisagé. La pneumonie virale doit être considérée avec suspicion chez ces sujets, car une infection associée au cancer bronchiogène peut simuler la pneumonie virale.

Le diagnostic de cancer bronchiogène n'offre habituellement aucune difficulté si le médecin envisage la possibilité de son existence. Tous les hommes de plus de quarante ans, qui ont été de grands fumeurs, devraient être soumis à la radiographie pulmonaire systématique, au moins tous les six mois. Toute opacité doit être considérée comme suspecte du point de vue cancer. Malheureusement, la bronchoscopie ne permet le diagnostic que dans un tiers des cas seulement. L'examen cytologique permet un diagnostic correct dans environ 68% des cas. Dans 20% des cas, il est nécessaire d'avoir recours à l'exploration thoracique pour obtenir un diagnostic.

Dans une série de 1457 cas, 789 ont été jugés opérables, 723 cas ont été soumis à l'exploration, dans 469 cas, la résection a été possible. Le taux de mortalité opératoire était de 19%. La résection palliative a été pratiquée dans 73% des cas.

Le fait que seulement 32% des cas se soient prêtés à la résection, au moment où ils ont été vus pour la première fois, était sans doute dû au long délai, en moyenne, 8,7 mois, entre l'apparition des premiers symptômes et l'instauration du traitement.

Au bout de cinq ans, 13,5% des patients ayant subi les résections étaient encore vivants et paraissaient libérés du cancer. Etant donné que 85% seulement de la population générale exempts de cancer à l'âge de 60 à 70 ans sont encore vivants, les résultats sont meilleurs qu'ils n'apparaissent. Parmi les patients présentant des lésions étendues, et chez lesquels la résection palliative avait été pratiquée, 6,6% étaient vivants au bout de cinq ans. Dans un groupe de patients, chez lesquels aucune résection n'avait été faite, 17% ont survécu six mois et 1% seulement étaient vivants au bout de deux ans, même lorsqu'un autre traitement palliatif, tel que le chlorhydrate de NH_3 (moutarde-azote) ou l'irradiation, était appliqué.

Dans le cancer bronchiogène, il est évident que le taux de préservation ne saurait être accru que par un diagnostic plus précoce, ceci ne peut être accompli que si les grands fumeurs se soumettent à l'examen radiographique systématique, au moins tous les six mois.

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MODE OF ACTION OF ISONIAZID[1,2]

Part II

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In a preliminary paper on the mode of action of isoniazid (1), it was reported that tubercle bacilli susceptible to the drug became radioactive when incubated with C^{14} labeled isoniazid.[3] However, isoniazid-resistant tubercle bacilli did not become radioactive when incubated with C^{14} isoniazid. This suggested that fixation of isoniazid to tubercle bacilli is associated with inhibition of growth.

The present report is concerned with further studies on the fixation of isoniazid by tubercle bacilli under a variety of metabolic states and as a function of the drug's concentration in the medium.

MATERIALS AND METHOD

The same method was used in all of the experiments described to measure the fixation of isoniazid to the bacterial cell. Standard laboratory strains of H37Rv or R1Rv were incubated for ten to fourteen days in Dubos liquid Tween®-albumin medium (2). C^{14} labeled isoniazid synthesized by Murray and Langham (3), with the C^{14} in the carboxyl position, was then added to the cultures, usually in a concentration of 0.4 γ per ml., and the cultures were incubated for a further seven days.

The cultures were heated at 60°C. for thirty minutes, and the cells were separated from the medium by centrifugation. The organisms were washed three times with water containing 0.02 per cent Tween® 80 and finally suspended in water with 0.02 per cent Tween® 80. One milliliter amounts of this suspension, containing 1.5 to 3 mg. dry weight of organisms, were spread on weighed copper planchets 10 sq. cm. in area, and the radioactivity was measured in an internal continuous gas flow counter (4). If the weight of organisms plated was kept below 3 mg., no correction had to be made for self-absorption.

Measurements were made of the radioactivity of organisms before and after pasteurization, and it was found that heating the cultures to 60°C. for thirty minutes before washing and plating them did not alter their radioactivity. Subsequently, therefore, as a safety precaution the cultures were routinely pasteurized before the organisms were washed.

EXPERIMENTAL RESULTS

Effect of nicotinic acid, nicotinamide, and isonicotinic acid: To determine if tubercle bacilli would bind and retain compounds that resemble isoniazid in chemical structure, three C^{14} labeled compounds were tested in conjunction with C^{14} labeled isoniazid, and the relative uptake of radioactivity by the organisms is shown in figure 1.

There was no significant difference in radioactivity between isoniazid-susceptible and isoniazid-resistant bacilli incubated with C^{14} labeled nicotinic acid

[1] From the Department of Medicine, University of Chicago, Chicago, Illinois.

[2] This investigation was supported by a research grant (E-11) from the National Microbiological Institute, of the National Institutes of Health, Public Health Service. It was also aided in part by a grant from Motorola, Inc.

[3] The C^{14} used for the synthesis of this compound was obtained on allocation from the Isotope Division, United States Atomic Energy Commission.

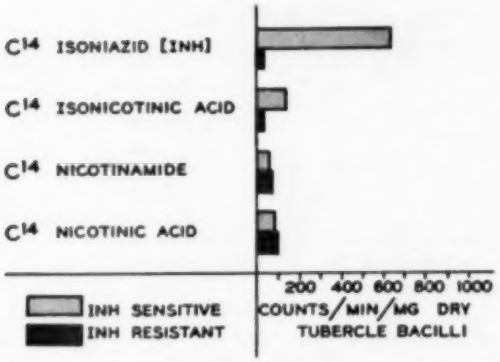

FIG. 1. Binding of radioactive drugs by tubercle bacilli

TABLE 1

EFFECT OF HEAT AND FORMALIN ON THE BINDING OF ISONIAZID BY TUBERCLE BACILLI

	Radioactivity	Weight of Isoniazid Bound
	cpm/mg	$\gamma \times 10^{-4}/mg$
Control	380	54
Heat-killed	30	4
Formalin-killed	30	4

H37Rv incubated seven days with 0.04 γ per ml. (0.019 μc per ml.) of C^{14} isoniazid.
Cpm/mg—counts per minute per mg. dry weight of bacilli.
$\gamma \times 10^{-4}$/mg.—micrograms $\times 10^{-4}$ of isoniazid adsorbed per mg. dry weight of bacilli.

and C^{14} labeled nicotinamide. The small amount of radioactivity of the isoniazid-susceptible organisms incubated with C^{14} isonicotinic acid was presumably a result of traces of C^{14} isoniazid which were found by paper chromatography as a contaminant of the preparation of isonicotinic acid. The difference in radioactivity between isoniazid-susceptible and isoniazid-resistant organisms incubated with C^{14} labeled isoniazid was large and occurred only with isoniazid and not with the other three substances tested.

Effects of heat and formalin on bacilli: Whether or not bacilli killed by exposure to 60°C. for thirty minutes or by exposure to 1 per cent formalin could subsequently bind isoniazid was examined. Fourteen-day-old cultures of H37Rv in Dubos liquid Tween®-albumin medium were killed by either heating to 60°C. or by exposure to 1 per cent formalin and were then exposed to C^{14} isoniazid for seven days. The uptake of radioactivity is shown in table 1. Neither the heat-killed nor the formalin-killed bacilli retained the isoniazid.

Absorption of metabolically inactive bacilli: To assess whether or not living but metabolically inactive cells could fix isoniazid, the metabolism of the cultures was arrested by either prior exposure to streptomycin or by keeping the culture at 5°C. after adding the labeled isoniazid.

TABLE 2

BINDING OF ISONIAZID BY METABOLICALLY INACTIVE BACILLI

	Radioactivity	Weight of Isoniazid Bound
	cpm/mg	$\gamma \times 10^{-4}$/mg
C^{14} isoniazid at 37°C	800	114
Streptomycin, then C^{14} isoniazid	1,400	200
C^{14} isoniazid at 5°C	1,270	181

H37Rv exposed for seven days to 1 γ per ml. (0.0488 μc/ml.)
$\gamma \times 10^{-4}$/mg. = micrograms $\times 10^{-4}$ of isoniazid absorbed per mg. dry weight of bacilli.

TABLE 3

EFFECT OF MULTIPLE-DRUG TREATMENT ON THE BINDING OF ISONIAZID BY TUBERCLE BACILLI

	Radioactivity	Weight of Isoniazid Bound
	cpm/mg	$\gamma \times 10^{-4}$/mg
C^{14} isoniazid 37°C	300	43
C^{14} isoniazid 5°C	690	94
C^{14} isoniazid–streptomycin	620	89
C^{14} isoniazid–streptomycin–PAS	640	91

R1Rv exposed for seven days to 0.5 γ per ml. (0.0244 μc per ml.) of C^{14} labeled isoniazid.

Arrest of metabolism either by streptomycin or low temperature resulted in a greater binding of isoniazid than occurred in organisms maintained under optimal metabolic conditions (table 2).

Effect of combined antimicrobial treatment: As organisms maintained under unfavorable growth conditions or organisms previously inhibited by streptomycin bound more isoniazid than did metabolizing bacilli, it seemed probable that simultaneous exposure to two drugs might also result in a greater fixation of the drug.

Fourteen-day-old cultures were exposed to C^{14} labeled isoniazid alone, and to pairings of isoniazid with streptomycin or para-aminosalicylic acid (PAS). The organism treated with the combination of drugs bound more isoniazid than did the control cultures and bound the same amount of drug as bacilli whose metabolism was arrested by exposure to cold (table 3).

C^{14} labeled isoniazid was used in several experiments in combination with PAS, but no consistent results were obtained. Sometimes the organisms were found to contain twice as much radioactivity as the control organisms incubated with C^{14} isoniazid alone. In other experiments, the uptake of isoniazid in the presence of PAS was only slightly greater than in the control organisms. Therefore, the results with the combination of PAS and C^{14} labeled isoniazid were not included in table 3.

Rate of fixation of isoniazid: To determine how rapidly isoniazid became bound to the bacilli, cultures of H37Rv were grown in 500 ml. of Dubos liquid Tween®-

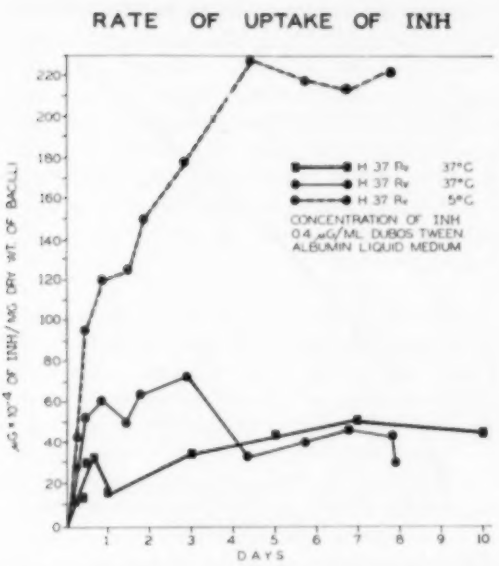
RATE OF UPTAKE OF INH
H 37 Rv 37°C
H 37 Rv 37°C
H 37 Rv 5°C
CONCENTRATION OF INH
0.4 γG/ML DUBOS TWEEN
ALBUMIN LIQUID MEDIUM
γG • 10⁻⁴ OF INH/MG DRY WT. OF BACILLI
DAYS

FIG. 2. Rate of binding of isoniazid by tubercle bacilli

TABLE 4

EFFECT OF THE CONCENTRATION OF ISONIAZID IN THE MEDIUM ON THE AMOUNT OF ISONIAZID BOUND BY TUBERCLE BACILLI

γ of Isoniazid Per Ml. of Medium	0.01	0.05	0.10	0.20	0.24	0.40	2.95	5.82	11.32	21.4
γ × 10^{-4} absorbed	3.4	10	21	30	41	54	243	366	491	714

H37Rv exposed for seven days to C^{14} labeled isoniazid at varying concentrations.
γ × 10^{-4} absorbed per mg. dry weight of bacilli.

albumin medium in a 1,000 ml. flask with a side arm. The side arm was fitted with a ground glass joint that accepted its counterpart on 125 ml. Erlenmeyer flasks. Aliquots of the culture could then be tilted out of the large flask into the small Erlenmeyer flasks at any desired intervals. The bacilli in these aliquots were washed and counted as described under MATERIALS AND METHODS.

Figure 2 shows the uptake of C^{14} isoniazid by a strain of H37Rv susceptible to the drug. The graph illustrates once again that more isoniazid became fixed to the cells at 5°C. than at 37°C. Moreover, it shows that the initial rate of fixation was just as rapid at 5° as at 37°C. The initial uptake was very rapid, reaching a peak at approximately sixteen to twenty-four hours. Following this original peak, a drop occurred in the amount of drug bound to the cell. This drop occurred in repeated experiments but to a varying degree. The drop was as much as 50 per cent of the peak value or as little as 20 per cent of the amount originally bound to the

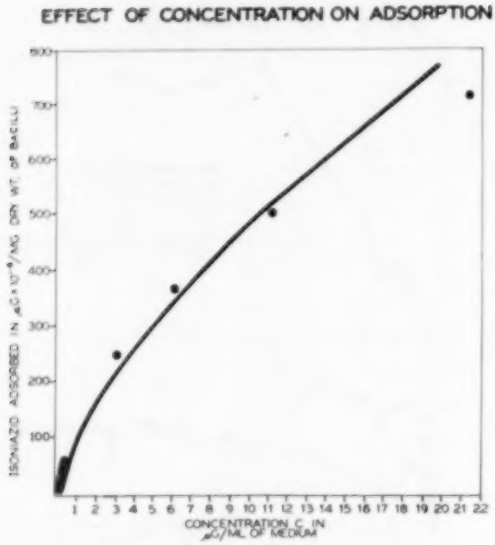
EFFECT OF CONCENTRATION ON ADSORPTION
ISONIAZID ADSORBED IN μG x 10⁻³/MG DRY WT. OF BACILLI
100
200
300
400
500
600
700
800
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
CONCENTRATION C IN μG/ML OF MEDIUM

FIG. 3. Relationship between the concentration of isoniazid in the medium and the binding of isoniazid by tubercle bacilli.

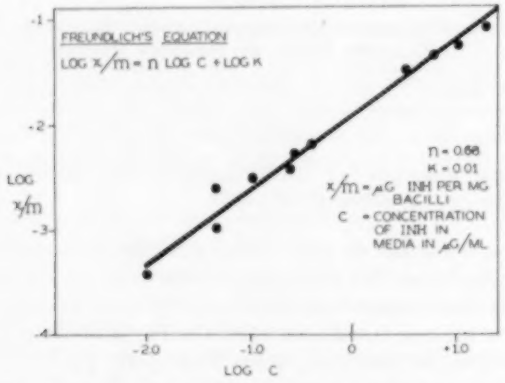

FIG. 4. Concentration of isoniazid in the medium related to the binding of isoniazid to tubercle bacilli according to Freundlich's equation.

cells. Whether this decline in the amount of drug fixed to the cells represents a relative increase in the cell mass during this period or actual loss of isoniazid that had been bound to the cells is not clear. Following this drop, the curve rose in an irregular fashion, usually reaching a plateau by the end of seven days, at a time when most of the organisms had lost their acid-fastness (5).

Amount of isoniazid bound as a function of its concentration in the surrounding medium: Two-week-old cultures of H37Rv were exposed to concentrations of C^{14} labeled isoniazid ranging from 0.01 γ per ml. to 20.7 γ per ml., and the amount of isoniazid bound is shown in table 4 and figure 3.

The shape of the curve in figure 3 resembles that relating the adsorption of one substance by another. Applying an empirical equation for adsorption to the data in table 4 gives a straight line as shown in figure 4. Such correlation only suggests adsorption as a possible mechanism for the binding of isoniazid. This could occur on the cell surface or could take place on components within the cell.

DISCUSSION

Fixation of a chemotherapeutic agent to susceptible cells is not an isolated phenomenon. Ehrlich originally suggested that a drug was effective according to the degree it was bound to the parasite cell. In the case of arsenosa compounds, Eagle and Magnuson (6) showed a proportional relationship between the binding of the compounds by trypanosomes and their trypanocidal activity. Eagle (7) also has shown a relationship between the cytotoxic action of penicillin and the amount of penicillin bound to the affected cells. Therefore, one may conclude that binding of certain chemotherapeutic agents to cells is an important step in their mechanism of action. Information concerning the dynamics of the binding process and the site of fixation are prerequisites to an understanding of the mode of action of isoniazid. Although both isoniazid and penicillin exert their cytotoxic effects by first being bound to the susceptible bacterial cell, the binding process for these two drugs differs in several important respects.

In the case of tubercle bacilli, the binding agent is extremely susceptible to heat and to chemical agents and its properties are destroyed by heating the cells to 60°C for thirty minutes or by 1 per cent formalin. The substance responsible for binding penicillin, however, is thermostable and chemically stable to a high degree. Treatment of staphylococci by autoclaving or with 3 per cent formalin does not block the site of penicillin attachment (8).

Isoniazid fixation by tubercle bacilli is greater in amount if the organisms are in a resting state or maintained at a low temperature. However, penicillin fixation is less in amount in the case of resting organisms and is decidedly greater in actively growing cultures (9, 10).

The two drugs also differ with respect to rate of fixation. Penicillin is rapidly bound, and in the case of resting organisms the maximal amount is bound in three minutes (9). The binding reaction for isoniazid, however, does not reach an initial peak until at least sixteen hours of contact with the bacteria has been established, and the amount bound does not reach constant levels for seven to ten days.

A marked difference exists between isoniazid and penicillin binding with respect to the effect of drug concentration in the surrounding medium. The curve for isoniazid fixation suggests adsorption of the drug, whereas the curves pertaining to penicillin (8) show two components, a specific binding and nonspecific binding capacity of the cells.

The type of drug fixation which occurs with penicillin strongly suggests that some essential substance, probably an enzyme, present in minute amounts in the cell combines with the penicillin and is thereby inactivated (10). Such a hypothesis, however, does not account for isoniazid's mode of action.

There are three possible mechanisms by which a drug can become bound to susceptible cells: chemical combination with active groups in the cell; enzyme-dependent reactions in which the drug substitutes for a moiety within an essential metabolite or enzyme system, e.g., the suggestion of Zatman and associates (11) that isoniazid exchanges with nicotinamide in diphosphopyridine nucleotide (DPN) in the presence of certain diphosphopyridine nucleotidases; and physical or physicochemical adsorption.

If isoniazid combined chemically with active groups in the cell, the reaction rate would be governed by temperature. Since the binding process takes place at approximately the same speed at 5°C. as it does at 37°C., then it is not likely an ordinary chemical reaction.

An enzyme-initiated reaction likewise would proceed more slowly at low than at higher temperatures, although it would eventually proceed more to completion at the lower temperature. Moreover, if an essential enzyme or metabolite were inactivated by combination with isoniazid, then the binding of large quantities of isoniazid would arrest metabolism more rapidly than would the binding of smaller quantities of isoniazid. This does not hold, however, for the bacilli behave in the same fashion if minimal inhibiting doses of isoniazid are added to the medium or if large doses are added (1).

From the data presented here on isoniazid binding and comparison with data obtained by others on penicillin binding, it seems likely that the two mechanisms are quite different and that isoniazid becomes fixed to the cell by a process of physicochemical adsorption. The adsorption of isoniazid does not immediately curtail the organisms' metabolism but inhibits sustained and continued metabolism either by permitting accumulation of substances which become toxic to the cell or by interfering with the reactivation of enzymes concerned with hydrogen transport.

SUMMARY

The fixation of C^{14} labeled isoniazid to tubercle bacilli under varying metabolic states of the organisms has been described. Tubercle bacilli do not bind C^{14} labeled nicotinic acid, nicotinamide, or isonicotinic acid in any significant amounts. Heat-killed or formalin-killed bacilli do not bind isoniazid. More isoniazid is bound by cells whose metabolism is arrested by suboptimal temperatures or by streptomycin and PAS than is bound by cells growing under optimal conditions. Maximum binding of the drug takes at least sixteen to twenty-four hours. This initial peak is followed by a drop in the amount bound, and then further fixation of the drug proceeds for another seven to ten days. The relationship between the amount of isoniazid bound to the cells and the concentration of drug in the medium can be expressed by Freundlich's equation for physical adsorption. These results indicate that the fixation of isoniazid to susceptible

tubercle bacilli is governed by a mechanism of a different sort than that which governs the fixation of penicillin to penicillin-susceptible organisms.

SUMARIO

Modo de Acción de la Isoniacida: Parte II

Descríbese la fijación de la isoniacida rotulada C^{14} a los bacilos tuberculosos en distintos estados metabólicos de los microbios. Los bacilos tuberculosos no fijan el ácido nicotínico, la nicotinamida o el ácido isonicotínico, rotulados C^{14}, en cantidades de mayor importancia. Los bacilos matados al calor o con formalina no fijan la isoniacida. Fijan más isoniacida las células cuyo metabolismo es estacionado por temperaturas subóptimas o por estreptomicina y PAS que las células que crecen en condiciones óptimas. La fijación máxima de la droga consume a lo menos de dieciséis a veinticuatro horas. Esa cúspide inicial va seguida de una baja de la cantidad fijada y luego prosigue de nuevo la fijación de la droga durante otros siete a diez días. Cabe expresar con la ecuación de Freundlich para la adsorción física la relación que existe entre la cantidad de isoniacida fijada a las células y la concentración de la droga en el medio. Indican esos resultados que la fijación de la isoniacida a los bacilos tuberculosos susceptibles se rige por un mecanismo de distinto género que el que gobierna la fijación de la penicilina a microbios penicilinosusceptibles.

RESUME

Mode d'action de l'isoniazide: IIème Partie.

Description de la fixation de l'isoniazide marquée C^{14} sur le bacille tuberculeux dans diverses conditions métaboliques du bacille. Les bacilles tuberculeux ne fixent pas l'acide nicotinique, l'amide nicotinique ou l'acide isonicotinique en quantités significatives. Les bacilles tués par la chaleur ou par le formol ne fixent pas l'isoniazide. La quantité fixée par les cellules dont le métabolisme est réduit par une température suboptima ou par la streptomycine et le PAS est plus importante que celle fixée par les cellules se développa dans des conditions plus favorables. Il faut au moins seize à vingt-quatre heures pour observer le maximum de fixation du composé. Le commet initial de fixation est suivi d'une chute de la quantité fixée, puis d'une nouvelle période d'élévation de la fixation pendant encore sept à dix jours. Le rapport entre la quantité d'isoniazide fixée par les cellules et la concentration de la drogue dans le milieu peut s'exprimer par l'équation de Freundlich pour l'absorption physique. Ces résultats indiquent que la fixation de l'isonizaide par les bacilles sensibles est gouvernée par un mécanisme différant de celui qui règle la fixation de la pénicilline par les germes sensibles.

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THE RESPONSE OF THE RETICULO-ENDOTHELIAL SYSTEM TO THE INJECTION OF THE "PURIFIED WAX" AND THE LIPOPOLYSACCHARIDE OF TUBERCLE BACILLI[1,2]

A Histologic and an Immunologic Study

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INTRODUCTION

Raffel and associates (1, 2) have shown the important role played by the wax fraction of the tubercle bacillus in the mechanism of production of the delayed type of hypersensitivity. The wax fraction, or, more strictly, the lipopolysaccharide isolated from it by Asselineau and Lederer (3, 4), has been found essential for the induction of the delayed hypersensitivity not only to tuberculin or tuberculoprotein but also to antigens without relation to tubercle bacilli, such as ovalbumin or picryl chloride (2, 5). From these studies it was concluded that delayed hypersensitivity and immunity in tuberculosis were two separate entities (4, 6). This view was supported by the demonstration that only guinea pigs which had received BCG showed any demonstrable protection against a subsequent infection with a virulent strain, whereas guinea pigs prepared with heat-killed bacilli or with the wax fraction were not protected in spite of the fact that the former reacted strongly to tuberculin.

No information is available concerning the mechanism whereby tubercle bacillary wax or its contained lipopolysaccharide changes the host to the simultaneously injected antigen so as to result in the development of the delayed rather than the anaphylactic type of hypersensitivity. Typical of the first is the absence of correlation between circulating antibody and responsiveness of the skin or the body cells. The second type has all the characteristics of a reaction depending on the existence of humoral antibody.

Thus, it seems reasonable to assume that the wax or lipopolysaccharide directs the immunologic mechanism along a channel different from that resulting in the elaboration of humoral antibody. On the other hand, tubercle bacilli are a constituent of the well-known adjuvant mixtures, and, moreover, it has been repeatedly demonstrated that their omission from such a system greatly reduces the efficiency of the mixtures as adjuvants (7, 8).

If this adjuvant action was the property of the same wax fraction of tubercle bacilli, then the facts seem incompatible with the simple concept of the separateness of immunity and hypersensitivity. In the hope of elucidating these ambigui-

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ties, one of the present writers studied the reaction of the antibody-forming system to the injection of antigen (ovalbumin) in water-in-oil emulsion with and without the addition of the wax fraction of tubercle bacilli (9). Briefly summarizing, it was found that the presence of the "purified wax" fraction (Anderson) in water-in-oil emulsions of antigen greatly potentiates the humoral antibody responses to the latter. The morphologic accompaniment of this increased antibody production, and upon which it seemed to depend, was a stimulation of derivatives of the plasma cellular series in certain draining lymph nodes and also in situations such as the spleen and liver. But the most impressive cellular event which followed the incorporation of the "purified wax" fraction with the water-in-oil emulsion of the antigen was the enormous proliferation of macrophages which occurred both locally at the site of injection, in draining lymph nodes and remote organs like the lungs and spleen. Techniques for staining antibody failed to reveal an antibody content of these cells.

In view of the striking morphologic consequences of the impact of the tubercle bacillary wax upon the macrophage system of the body, it was decided to determine whether the latter had developed any altered reactivity with regard to living tubercle bacilli when exposed to them in tissue cultures. It has previously been shown (10) that the monocytes from guinea pigs vaccinated with BCG did inhibit intracellular multiplication of tubercle bacilli. This inhibition of growth was found to be a property of the cells and was independent of any humoral factor.

It is further the purpose of this paper to detail similar experiments in which the lipopolysaccharide fraction of tubercle bacilli, supplied by Lederer, was used instead of wax in this respect, and also to describe briefly the morphologic consequences of the injection of such lipopolysaccharide in water-in-oil emulsion of antigen.

MATERIALS AND METHODS

Animals: Female guinea pigs, albinos or white animals, were obtained from outside sources. The animals weighed approximately 500 gm. when first used for the experiments. They were fed upon a pellet diet, with cabbage *ad libitum*.

Fractions of tubercle bacilli:

(*a*) *"Purified wax" fraction:* The wax used in this study was supplied by Dr. Rudolf Anderson,[4] and consisted of "Purified Wax" (Anderson (11)). The initial stages in its preparation involve extraction of tubercle bacilli with alcohol-ether followed by chloroform extraction of the bacillary residues. The "crude wax" obtained by these operations is separated (12) into two fractions by precipitation thrice from ether solution by cold methyl alcohol and twice from toluene by methyl alcohol and acetone. The principal product is "purified wax," while "soft wax" remains in solution.

(*b*) *Lipopolysaccharide:* This fraction consists of 'Lipopolysaccharide Test', and was prepared by Dr. Lederer[5] from "purified wax" by chromatographic separation on alumina columns. It consists primarily of a polysaccharide ester of mycolic acid (3).

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[4] We are very grateful to Dr. Rudolph J. Anderson, Yale University, for the supply of the "purified wax," which was sent to Dr. Albert H. Coons.

[5] This material was prepared by Dr. Edgar Lederer, Institute de Biologie-Chimique, Paris 5e, and was kindly supplied by Dr. Sidney Raffel, Department of Bacteriology and Experimental Pathology, Stanford University, School of Medicine, Stanford, California.

Tubercle bacilli: The BCG strain was obtained through the courtesy of Dr. J. D. Aronson from The Henry Phipps Institute in Philadelphia, Pennsylvania.

Preparation of the water-in-oil emulsion: The emulsion consisted of saline or, in the earlier experiments, of saline solution of ovalbumin; Arlacel (Batch 3375, Atlas Powder Company, Wilmington, Delaware) and Bayol F (Esso Standard Oil Company) in the proportions of 1:1:3 by volume. The details of the preparation are given in a previous publication (9). When the tubercle bacillary wax or lipopolysaccharide was used, this was dissolved in the Bayol F before emulsification. The guinea pigs received a single injection of 0.2 ml. of these emulsions subcutaneously in the footpad of the left hind leg.

Tuberculin tests: The guinea pigs were tested for skin hypersensitivity to tuberculin fourteen to sixteen days after injection of the fractions. One-tenth of a milliliter of a solution containing 0.1 mg. of Old Tuberculin was injected intracutaneously. The Old Tuberculin was supplied and prepared by The Massachusetts Department of Public Health, Biologic Laboratories, Forest Hills, Massachusetts. The reactions were read twenty-four and forty-eight hours after injection.

Tissue cultures of macrophages: The tissue culture technique has been described in an earlier paper (13). The procedure will be briefly outlined in the following:

The guinea pigs were injected intraperitoneally with 10 ml. of a solution of physiologic saline containing 0.01 mg. of glycogen per ml. Five days later the animals were bled from the heart. The exudates, consisting mainly of mononuclear phagocytes, were collected by introducing into the peritoneal cavity aseptically 20 ml. of balanced salt solution (Hanks) with 1:20,000 heparin. The fluid was then withdrawn and kept at room temperature. Aliquots of 9 ml. of exudate were adjusted to contain 1,500 to 2,000 white cells per cu. mm., and 1 ml. of a 1:10 dilution of a centrifuged culture of tubercle bacilli grown in Dubos Tween®-albumin medium was added. The mixture was then poured into a small Petri dish in which ten pieces of coverslips (7 × 15 mm.) had been placed on the bottom. The dish was incubated for one hour at 37°C.

During this time the cells settled on the coverslips and phagocytized tubercle bacilli. The coverslips were taken out singly, washed in balanced salt solution, and covered with a membrane of formvar. They were washed again and placed singly in small screw-capped vials containing 0.5 ml. of liquid culture medium. The cultures were kept at 37°C. in an almost horizontal position to keep the coverslips with the cells on the upper side completely covered by the medium. The medium consisted of balanced salt solution, 30 per cent normal guinea pig serum, and contained 2.5 γ of streptomycin and 50 units of penicillin per ml. Duplicate cultures were taken out after twenty-four hours' to seven days' incubation. The cells were fixed with methyl alcohol and stained with carbolfuchsin and Giemsa.

In each culture, 100 phagocytes containing tubercle bacilli were counted and were classified, according to the number of tubercle bacilli which they contained, in one of the following four groups: cells containing 1 to 2 bacilli, 3 to 5, 6 to 10, and those containing more than 10 bacilli. As demonstrated in a previous paper (13), an increase of the number of tubercle bacilli within the phagocytic cells is an indication for intracellular multiplication.

RESULTS

Multiplication of Tubercle Bacilli Within Macrophages from Animals Injected Previously with the "Purified Wax" Fraction or the Lipopolysaccharide of Tubercle Bacilli

A short word of explanation here may prevent confusion regarding the use of albumin in the early experiments of this study. The original idea was to find out whether mononuclear phagocytes from animals which had acquired delayed hypersensitivity to egg albumin, as a consequence of the injection of egg albumin together with the purified wax, would inhibit intracellular multiplication of tu-

bercle bacilli when cultivated in presence of the antigen to which they were sensitized. As it was found that the cultured cells, even without the antigen, did inhibit intracellular multiplication, an attempt was made in subsequent experiments to concentrate on the effect of the "purified wax" and of the lipopolysaccharide alone. This mode of approach to the problem also explains why the "purified wax" and lipopolysaccharides were used as "water-in-oil" emulsions, although presumably the water phase is not necessary for their action. For the present it is convenient to retain this mode of preparation of sensitizing mixtures since it is proposed to study subsequently the influence of added antigenic substances in the water phase.

Experiment I: The animals of this first series were injected with 10 mg. of ovalbumin as a water-in-oil emulsion, with or without the addition of the wax fraction of tubercle bacilli. Three days before autopsy at twenty-one days after injection, these animals were subjected to skin tests and corneal tests.

The results indicated that both the immediate and delayed types of hypersensitivity to ovalbumin were present. The results of a small-scale tissue culture experiment demonstrated that the macrophages derived from guinea pigs which had been injected with water-in-oil emulsion with tubercle bacillary wax or with the same plus ovalbumin inhibited the multiplication of tubercle bacilli. The controls were animals injected with ovalbumin in water-in-oil emulsion without tubercle bacillary wax, and the macrophages from these animals showed no evidence of such a refractory state toward tubercle bacilli. Ovalbumin (0.5 mg. per ml.) was added to the medium of some of the cultures. Further experience demonstrated that this addition was without influence upon the interaction between phagocytes and tubercle bacilli under the conditions of these experiments.

It should be noted that this technique of cultivating mononuclear phagocytes does not allow any judgment of their migrating activities. Therefore, a possible cytotoxic effect of egg albumin on these sensitized cells could not be observed.

Experiment II: In a second experiment, 12 guinea pigs were prepared and all were injected with water-in-oil emulsion, either with no addition or with addition of 10 mg. of ovalbumin, or of "purified wax" fraction in various doses, or of both ovalbumin and "purified wax" fraction.

Eighteen days after injection, all animals were tested for skin hypersensitivity to tuberculin. The guinea pigs prepared with ovalbumin or with the emulsifying agent alone were negative. All animals which had been injected with the wax showed a weak reaction not exceeding a diameter of 10 mm., and all reactions began to recede after twenty-four hours. For each single tissue culture experiment, cells from 3 to 4 guinea pigs were used. The first group of animals was used twenty-four days after the injection, the second twenty-six days, the third thirty-two days, and the last group was done on the thirty-eighth day.

The results of some of these experiments are given in figure 1. Tests done with cells from the individual animals from the same group yielded almost identical values indicating multiplication or inhibition of tubercle bacilli.

Thus, monocytes derived from guinea pigs injected with 1.0 or 0.2 mg. of the wax fraction alone, or combined with ovalbumin, depressed bacillary mul-

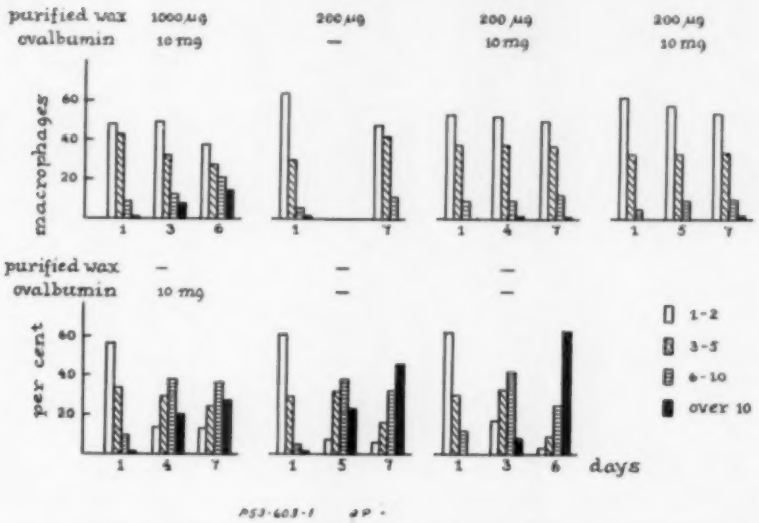

FIG. 1. Multiplication of tubercle bacilli (BCG) within cultivated macrophages from guinea pigs which had received a single injection of the "purified wax" fraction of tubercle bacilli. All animals were injected with the water-in-oil emulsion with the exception of the last one, which had no injection. The experiments were done twenty-four to thirty-eight days after the injections. The columns represent the percentage of phagocytes containing 1 to 2, 3 to 5, 6 to 10, or more than 10 tubercle bacilli. The days indicate how long the cells had been cultivated.

tiplication to a considerable extent. By contrast, multiplication of tubercle bacilli was observed within the monocytes from the control animals or those animals injected with ovalbumin or with the emulsifying agent. The extent of inhibition of intracellular multiplication of tubercle bacilli by monocytes from the animals injected with the "purified wax" in water-in-oil emulsion is similar to the inhibition found after vaccination with BCG (10). A slight difference of the inhibitory power of the macrophages can be noticed between cells derived from animals which had been prepared with 1,000 γ of the "purified wax" and those injected with 200 γ.

Experiment III: In a third experiment, *wax fraction* and *lipopolysaccharide* were prepared as water-in-oil emulsions and three groups of 3 animals each were injected subcutaneously with 0.2 ml. of the following mixtures: *Group A:* saline emulsion in oil; *Group B:* 0.2 mg. of wax added to saline emulsion in oil; and *Group C:* 0.2 mg. of lipopolysaccharide added to saline emulsion in oil. The animals of *Groups B* and *C* uniformly produced an increasing swelling of the feet, which led to desquamation of the epidermis and to ulceration. Hyperplasia of the regional lymph nodes became apparent from the seventh day on and increased steadily. These alterations were somewhat more pronounced in the guinea pigs of *Group C*, the animals injected with the lipopolysaccharide.

Tuberculin skin tests were done on the fourteenth day, and the dimensions of

TABLE 1

INTENSITY OF SKIN REACTIONS TO 0.1 MG. OF OLD TUBERCULIN IN GUINEA PIGS FOURTEEN DAYS AFTER INJECTION OF THE "PURIFIED WAX" AND THE LIPOPOLYSACCHARIDE FRACTIONS OF TUBERCLE BACILLI IN WATER-IN-OIL EMULSIONS

Group	Animal	Diameter of Reaction in Mm.	
		24 Hours	48 Hours
A. Control	1	7	2
	2	6	3
	3	5	4
B. "Purified wax" (200 γ)	1	6	3
	2	7	6
	3	7	7
C. Lipopolysaccharide (200 γ)	1	9	7
	2	6	5
	3	9	5

the skin reactions are given in table 1. The 3 animals of *Group A*, the controls, were negative, whereas the animals of the two other groups showed a mild reaction which was persistent. It is doubtful, however, whether these animals could be regarded as tuberculin-positive.

One animal from each group was sacrificed twenty-two, twenty-four, and twenty-five days after injection, respectively, and peritoneal exudates were collected from them which were used for tissue cultures as described above. Tissues of these animals were fixed and sectioned for histologic investigation.

The results of the tissue culture experiments are given in figure 2. Exudates of one animal from each group were used in *Experiments I*, *II*, and *III*, respectively. As can be seen from the figures, the monocytes from animals which had been injected with "purified wax" in water-in-oil emulsion, as well as with the lipopolysaccharide in water-in-oil emulsion, inhibited multiplication of tubercle bacilli strain BCG. The inhibition achieved was of the same magnitude as that found with monocytes from guinea pigs vaccinated with BCG.

Effect of the Lipopolysaccharide from Tubercle Bacilli on the Reticulo-Endothelial System

The following description refers to guinea pigs from *Experiment III* at three weeks following the injection of 0.2 ml. of water-in-oil emulsion, with and without the addition of 200 γ of lipopolysaccharide. The guinea pigs are designated, respectively, as *lipopolysaccharide* and *control* animals.[6] The lesion resulting from the injection of the "purified wax" as a water-in-oil emulsion has been described previously (9).

Local lesion: The injected feet of the lipopolysaccharide animals were enlarged to several times normal size, and the plantar region was occupied by a firm mass enveloping the plantar tendons. By contrast, the lesion in control animals consisted of a slight increase in spongy subcutaneous tissue.

[6] We are indebted to Dr. A. M. Pappenheimer for his interest and assistance.

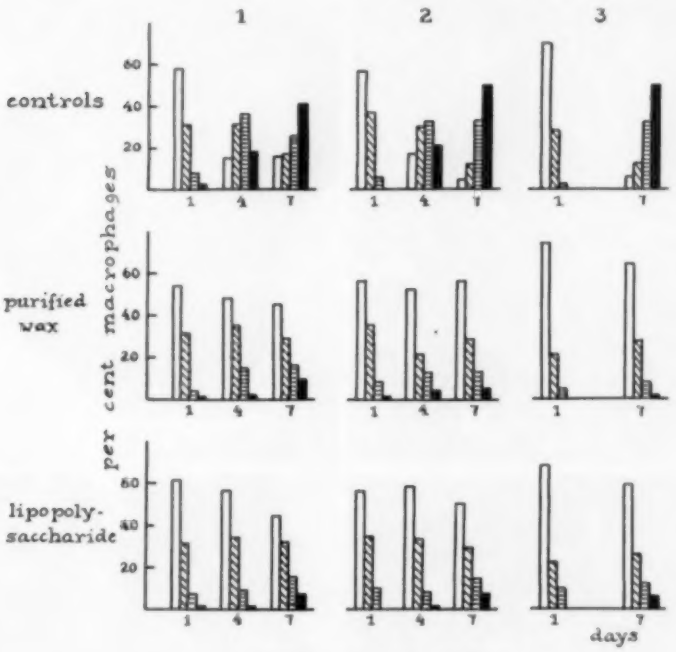

FIG. 2. Multiplication of tubercle bacilli (BCG) within cultured macrophages from guinea pigs injected previously with the "purified wax" or the lipopolysaccharide of tubercle bacilli. The injections had been given twenty-two to twenty-five days prior to the tissue culture experiments. For each experiment (*Experiments I, II,* and *III*), cells from one animal of each group (*A, B,* and *C*) were used simultaneously. For explanation *see* figure 1.

Sections of the control animals showed small collections of macrophages enclosed by collagenous tissue containing young fibroblasts. These macrophages were small in size. The nuclear membranes were delicate, and the interior of the nucleus was occupied by a delicate chromatin network with nucleoli. The cytoplasm was of small extent and faintly basophilic. Multinucleate giant cells were present. Areas of degenerating polymorphonuclear leukocytes and nuclear debris were also encountered.

The foot lesions of the lipopolysaccharide animals were mainly a proliferation of macrophage elements. As will be appreciated from figure 3*A*, these had a very different appearance from the macrophages of the control animals. The nucleus was round or oval. The nuclear membrane was transformed from the delicate crenated structure of the normal macrophage into a prominent thickened layer which surrounded a relatively "empty" interior. Nucleoli were large and prominent. The cytoplasm was bulky and possessed an opaque finely granular structure. The nucleus of these cells occupied an eccentric position at the periphery,

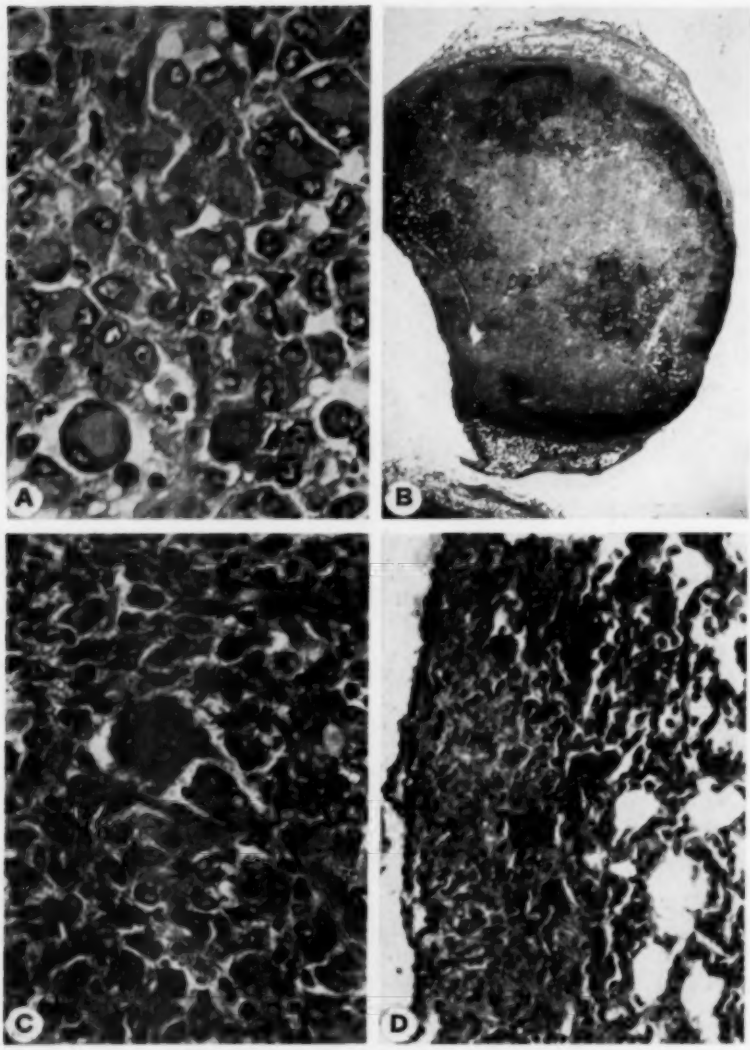

FIG. 3. All microphotographs were from tissues of a guinea pig injected with 200 γ of lipopolysaccharide in water-in-oil emulsion subcutaneously into the footpad and sacrificed after three weeks. The sections were stained with hematoxylin and eosin.

A. Subcutaneous tissue from footpad. Proliferation of macrophage elements with the cytologic changes described in the text. (×554).

B. Homolateral lymph node from flank. Note proliferation of pale tissue composed mainly of macrophage elements occupying medulla. The normal but compressed cortex shows as darker tissue in the periphery. (×8).

C. Detail from medulla of same lymph node as figure 3*B*. Proliferation of macrophage elements with giant cell formation. (×400)

D. Lung. Subpleural granuloma. (×205)

and the center of the cell was occupied by a circular eosinophilic area, often well demarcated from a basophilic periphery. Staining with Sudan III failed to demonstrate a cytoplasmic fat content. By staining with the Ziehl-Neelsen technique, no acid-fast material could be demonstrated within these cells. Foot's reticulum stain demonstrated an arrangement of loculi of 10 to 30 packed cells, the reticulin fibers passing around the loculi but failing to penetrate between the individual cells. Multinucleate giant cells were numerous. The macrophage elements were intermingled with varying numbers of polymorphonuclear neutrophil leukocytes and eosinophil leukocytes. Irregular spaces containing degenerating polymorphonuclear leukocytes and nuclear debris were also found.

Lymph nodes: The *control* animals showed a quite perceptible enlargement of the homolateral popliteal lymph node and of the nodes of the homolateral flank and inguinal regions, but this was of slight degree when compared with the enormous lymphatic enlargement present in animals receiving lipopolysaccharide. Thus, the popliteal nodes of the latter were up to five times the weight of the control, and two or three nodes in the homolateral flank region were enlarged so as to be up to eight times the weight of those of the *control* animals. Also, macroscopically, obvious enlargement was apparent in the homolateral inguinal lymph nodes and of nodes along the side of the inferior vena cava up to the renal veins. As seen in figures 3*B* and 3*C*, this enlargement was due, in the main, to a proliferation of macrophage elements, which completely filled the medulla and compressed the cortex so that only strands and islands of the latter remained visible. The cells composing this new tissue were of similar appearance to those described occupying the site of injection.

Liver: While no histologic abnormality was found in the livers from *control* animals, sections of the livers of *lipopolysaccharide* animals showed small granulomata in the portal spaces. These were composed of mixed cells: macrophages together with plasma cells, lymphocytes, and scanty polymorphonuclear leukocytes.

Lungs: All of the *lipopolysaccharide* animals demonstrated the presence of numerous grayish-white irregular areas 0.5 to 2 mm. in diameter on the pleural surface of the pulmonary lobes. Sections showed that these areas were granulomata in which the main cell type was the macrophage, or modifications of these to a cell similar to that described in the footpad. Multinucleate giant cells were common (figure 3*D*). While many of these granulomata were subpleural in situation, others were situated within the pleural lobes, especially in the peripheral parts of these.

Spleen: The medulla showed a striking proliferation of macrophage elements, especially within the sinuses, which were packed with cells and greatly expanded, but also groups of cells in the medullary cords.

In summary, these morphologic results demonstrated the existence in the animals which had received lipopolysaccharide of a widespread proliferation of the macrophage elements of the reticulo-endothelial system. The record of these results has focused attention on these particular cells. A full morphologic description of the lesions is not included in this communication since the results

are regarded as generally reproducing the cellular changes which follow injection of the "purified wax" fraction of tubercle bacilli (Anderson) as a water-in-oil emulsion and which have been recorded previously (9). When directly compared, as was done with *Group B* and *Group C* in the above experiment, 200 γ of lipopolysaccharide were found to produce more pronounced cellular changes than 200 γ of "purified wax" fraction.

DISCUSSION

The experiments reported in this paper were undertaken because it was thought that the generalized reaction of the reticulo-endothelial system of guinea pigs to the injection of the "purified wax" fraction might possibly be of importance for the emergence of an altered interaction between phagocytic cells and tubercle bacilli in the immune animal. The results indicate that monocytes, i.e., phagocytes which belong to the reticulo-endothelial system (14, 15) and probably contribute to the formation of the typical tuberculous lesion, acquire growth-inhibitory activity against tubercle bacilli. This dual response of the reticulo-endothelial system to the injection of the wax fraction or the lipopolysaccharide in a water-in-oil emulsion, formation of epithelioid cellular nodules and growth inhibitory property, seems of great interest.

It had long been known that dead tubercle bacilli could produce typical tuberculous lesions at a location distant from the site of injection provided that the bacilli were injected as an emulsion in a mineral oil; there was no other pathogenic activity of the dead bacilli beyond the local abscess formation if the mineral oil was omitted (16, 17). The same seems to be true for the "purified wax" fraction (9) or, as shown in this paper, for the lipopolysaccharide of tubercle bacilli. In the present experiments, in all of which the "purified wax" and the lipopolysaccharide were injected as a water-in-oil emulsion, lesions occurred not only at the site of injection but also in lymphatic nodes, the spleen, and the lungs. In rats, however, typical tubercles were found in the lungs after the intraperitoneal injection of dead tubercle bacilli without the addition of mineral oil (18). In reports by Raffel and associates (1, 2, 4, 5, 6), no systemic lesions were described either after the injection of whole heat-killed tubercle bacilli, the "purified wax," or the lipopolysaccharide. Thus, it seems reasonable to assume that the mineral oil, although not essential, contributes to the distribution of the active material and to the generalized reaction of the reticulo-endothelial system.

Generalization of the process may not be necessary for the elicitation of the delayed type of hypersensitivity (2), whereas it might be important for the induction of the growth-inhibiting activity of the monocytes. In all of the present experiments, in which cells from animals injected with either wax or lipopolysaccharide as water-in-oil emulsion were used for the tissue cultures, the monocytes suppressed intracellular multiplication of tubercle bacilli. These findings are in contrast to the results obtained in the intact animal (6), in which neither heat-killed tubercle bacilli nor any component thereof induced any increased resistance against a subsequent infection with virulent tubercle bacilli. But it is possible

that the use of the water-in-oil emulsion is essential for the induction of the growth-inhibitory property of phagocytes by the wax and lipopolysaccharide as well as of immunity by dead tubercle bacilli. This is indicated by the fact that increased resistance to the injection with virulent tubercle bacilli was obtained after the intraperitoneal injection of guinea pigs with dead tubercle bacilli in mineral oil (19).

Choucroun (20) observed lesions in the lungs of guinea pigs after the intraperitoneal injection of a small amount of a carbohydrate-lipid complex, the so-called toxic fraction, in paraffin oil. As a result of these injections, some animals showed increased resistance to the virulent infection. Unfortunately, there is no precise pathologic description of the lesions, which makes a comparison impossible. Furthermore, it is conceivable that the dosage of these materials determines the outcome of the experiment. This is indicated by the data presented in figure 1. It is apparent that macrophages from animals injected with 1,000 γ of wax fraction exhibited less inhibitory power than did the macrophages from animals injected with 200 γ.

This is not the place to discuss the chemistry and the biologic activity of lipoid components of the tubercle bacillus. Recent reports by Bloch and others on the isolation and identification of toxic fraction of the tubercle bacillus represent an important step toward a clarification of these problems (21).

Finally, it should be emphasized that the results reported in this paper do not prove that guinea pigs injected with either the "purified wax" fraction or the lipopolysaccharide derived from tubercle bacilli exhibit increased resistance against a subsequent injection with virulent tubercle bacilli. It can only be concluded that the macrophages from such animals when cultivated *in vitro* possessed the property of inhibiting intracellular multiplication of attenuated tubercle bacilli to a similar degree as did macrophages derived from animals vaccinated with BCG. The discrepancy between our results (10) and those recently obtained by Mackaness (22), using a somewhat different technique, cannot be explained at the moment. Factors possibly influencing the inhibitory power of cells from vaccinated animals have been mentioned briefly (23) and will be discussed later in detail when more experimental data are available.

SUMMARY

When 200 γ of the "purified wax" fraction (Anderson) of *M. tuberculosis* are injected into guinea pigs in water-in-oil emulsion, a systemic change is induced in the cells of the reticulo-endothelial system so that the monocytes derived from a peritoneal exudate are able to inhibit the growth of *M. tuberculosis* in tissue culture.

Two hundred micrograms of the lipopolysaccharide fraction (Lederer) of *M. tuberculosis* injected into guinea pigs in a water-in-oil emulsion are able to produce a systematized cellular reaction of the reticulo-endothelial system which closely resembles that following injection of "purified wax" fraction in water-in-oil emulsion, and the monocytes from peritoneal exudates of these animals, also, are able to inhibit the growth of *M. tuberculosis* in tissue culture.

SUMARIO

La Respuesta del Sistema Reticulo-Endotelial a la Inyección de la Cera Purificada y del Liposacárido de los Bacilos Tuberculosos. Estudio Histológico e Inmunológico

Cuando se inyectan 200 γ de la fracción de "cera purificada" (Anderson) del *M. tuberculosis* en cobayos en emulsión de agua-en-aceite, se induce una alteración orgánica en las células del sistema retículo-endotelial, de modo que los monocitos derivados de un exudado peritoneal son capaces de inhibir la proliferación del *M. tuberculosis* en cultivos en tejidos.

Doscientos microgramos de la fracción lipopolisacárida (Lederle) del *M. tuberculosis* inyectados en cobayos en una emulsión de agua-en-aceite son capaces de producir una reacción celular sistemática del sistema retículo-endotelial, que se parece mucho a la consecutiva a la inyección de la fracción de "cera purificada" en emulsión de agua-en-aceite, y los monocitos de los exudados peritoneales de esos animales son también capaces de inhibir la proliferación del *M. tuberculosis* en cultivos en tejidos.

RESUME

Réponse du système réticulo-endothélial à l'injection de cire purifiée et de liposaccharide extraits de bacilles tuberculeux. Etude histologique et immunologique

Quand 200 γ de la fraction "cire purifiée" (Anderson) de *M. tuberculosis* sont injectés au cobaye, sous la forme émulsion aqueuse dans l'huile, il se produit une modification particulière des cellules du système réticulo-endothélial, de telle sorte que les monocytes dérivés de l'exsudat péritonéal sont capables d'inhiber la croissance du *M. tuberculosis* dans la culture des tissus.

Deux cents microgrammes de la fraction liposaccharide (Lederer) de *M. tuberculosis*, injectés au cobaye dans une émulsion aqueuse dans l'huile, sont capables de déterminer une réaction tissulaire systématisée du système réticulo-endothélial, qui ressemble étroitement à celle qui succède à l'injection de la fraction "cire purifiée" en émulsion aqueuse dans l'huile. Les monocytes de l'exsudat péritonéal de ces animaux sont également capables d'inhiber la croissance du *M. tuberculosis* dans la culture des tissus.

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S-ETHYL-L-CYSTEINE, A MEMBER OF A NEW GROUP OF ANTITUBERCULOUS COMPOUNDS[1]

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INTRODUCTION

Since the discovery of the antituberculous activity of 4,4′-diaminodiphenyl sulfone in 1939 (1), a variety of synthetic compounds has been shown to possess *in vivo* activity. Para-aminosalicylic acid was the second type of synthetic compound to be described (2). Although possessing low activity when used alone, it retards development of resistance to streptomycin and isoniazid and has therefore been widely used in multiple-drug therapy. A further important advance was made by Domagk (3), who found that a series of thiosemicarbazones was highly active in experimental animals. Clinical trials revealed, however, that the thiosemicarbazones showed sufficient toxicity to limit their usefulness in man. In 1945, Chorine (4) found that niacinamide retarded the development of tuberculosis in the guinea pig and, independently, McKenzie and associates (5) showed that niacinamide was effective against tuberculosis in mice. Following from these observations, pyrazinamide (6, 7, 8) and shortly thereafter isoniazid were shown to possess antituberculous activity in the mouse (6, 7, 9*a*) and in man (8, 9*b*). Clinical and experimental studies (10, 11, 12, 13) have indicated that pyrazinamide and isoniazid together are more effective than either agent alone. Triton A-20, an alkyl aryl poleyther alcohol, is also active against tuberculosis in mice (14) and potentiates the action of streptomycin (15), but it has not been tested in man. More recently, antituberculous activity has been reported for thiocarbanilides (16).

Investigation of compounds in this laboratory for antituberculous activity has revealed that those possessing a thioethyl component may be efficacious in the mouse. The various categories of active compounds in this group have been noted by Brown and associates (17). During the course of the present studies, it was learned that Del Pianto (18) had observed activity for combinations of sodium ethyl thiosulfate with mercapto benzothiazole derivatives.

S-Ethyl-L-cysteine, a member of the series tested in the present studies, is a white, crystalline, water-soluble substance which is free of the offensive odor characteristic of thioethyl compounds and is well tolerated by experimental animals. It was more active than pyrazinamide or para-aminosalicylic acid, but less active than isoniazid when tested as a component of diet.

MATERIALS AND METHODS

Mycobacterium tuberculosis, human-type, strain H37Rv was used as the infecting organism for *in vivo* tests. Virulence of the culture has been maintained by serial passage of pellicle

[1] From the Merck Institute for Therapeutic Research, Rahway, New Jersey; and the Research Laboratories, Chemical Division, Merck & Co., Inc., Rahway, New Jersey.

growth in Proskauer-Beck medium. The culture for inoculation of test animals was prepared by growing pellicle in Dubos Tween®-albumin medium. After ten to fourteen days of incubation at 37°C., the cultures were adjusted with a Coleman 6A Spectrophotometer to permit 70 to 80 per cent transmission of light at 620 mμ. Barckmann IS-32 white mice weighing 15 to 17 gm. were inoculated intravenously with 0.25 ml. amounts of standardized cell suspension. Suspensions of *Mycobacterium tuberculosis* strains H37Rv resistant to dihydrostreptomycin, isoniazid, or para-aminosalicylic acid were prepared and utilized in a similar manner. Groups of 8 mice were used for each test dosage level.

For testing oral efficacy, S-ethyl-L-cysteine, para-aminosalicylic acid, or pyrazinamide were incorporated into the stock diet[2] and presented *ad libitum*. To determine efficacy by subcutaneous route, compounds were dissolved in distilled water at concentrations yielding the desired daily dose in 0.25 ml. of solution, and administered once daily five days per week beginning on the day after infection. To evaluate efficacy on the basis of gross pathologic involvement, the tests were continued until 50 per cent of the infected controls were dead. All of the animals which died during the course of the experiment as well as those which survived the test period were autopsied, and the lungs were observed for gross pathologic involvement with a 2× lens after forty-eight hours' fixation in 10 per cent formalin. The lung lesions were rated as follows:

0 = No involvement
1 = Minimal involvement, 10 per cent of tissue tuberculous
2 = Moderate involvement, 10–25 per cent of tissue tuberculous
3 = Moderate to advanced involvement, 25–50 per cent of tissue tuberculous
4 = Advanced involvement, more than 50 per cent of tissue tuberculous

For determination of efficacy by extension of survival time, dosing was continued until half of the infected controls were dead and the median survival time values (ST_{50}) were established by estimation from plots on logarithmic probability paper.

In vitro tests were performed both with Dubos Tween®-albumin medium and Youmans' modification of Proskauer-Beck medium. Solutions of S-ethyl-L-cysteine were sterilized by passage through a Seitz SK filter and 1 ml. amounts added aseptically to 9 ml. amounts of medium. The test cultures were inoculated with 0.1 ml. amounts of 1:10 dilutions of culture in Dubos Tween®-albumin medium adjusted to permit 90 per cent transmission of light at 620 mμ. The test series were observed for the presence of growth after fourteen days' incubation at 37°C.

RESULTS

S-Ethyl-L-cysteine was more effective than pyrazinamide when tested as a component of diet. The averaged results of four trials are shown in table 1. Both compounds were ineffective at 0.05 per cent concentration in the diet. At 0.1 per cent concentration in the diet, pyrazinamide was ineffective and S-ethyl-L-cysteine was moderately effective. At 0.2 per cent, S-ethyl-L-cysteine gave good to excellent effects as judged by the absence of grossly visible lesions, whereas pyrazinamide showed little or no efficacy. At concentrations of 0.35 per cent and 0.5 per cent in the diet, both compounds were highly effective. Similar experiments with isoniazid in this laboratory showed that a level of 0.005 per cent in diet produced complete suppression of gross pathologic lung involvement. Comparable results were obtained when extension of median

[2] S-2 diet: yellow corn meal, 31 per cent; whole wheat flour, 30 per cent; casein, technical, 10 per cent; soy bean meal, 10 per cent; linseed oil meal, 7 per cent; yeast, dried brewers, 5 per cent; alfalfa meal, 2 per cent; sodium chloride, 0.5 per cent; calcium carbonate, 0.5 per cent; Mazola oil, 3 per cent; cod liver oil, 1 per cent; and 10 mg. of vitamin B_2 per kg.

TABLE 1

EFFICACY OF S-ETHYL-L-CYSTEINE COMPARED WITH PYRAZINAMIDE

Per Cent Concentration in Diet	Average Lung Lesion Score	
	S-Ethyl-L-Cysteine	Pyrazinamide
0.05	3.9	4.0
0.10	1.9	3.8
0.20	0.9	3.1
0.35	0.0	0.1
0.50	0.1	0.1
Infected controls 0.0	4.0	

TABLE 2

COMPARISON OF ANTITUBERCULOUS ACTIVITY OF S-ETHYL-L-CYSTEINE, PYRAZINAMIDE, PARA-AMINOSALICYLIC ACID—EVALUATION OF EFFICACY BY EXTENSION OF MEDIAN SURVIVAL TIME

Compound	Per Cent Concentration in Diet			
	0.0	0.1	0.2	0.5
	Median Survival Time, Days			
S-Ethyl-L-cysteine	—	24	>40	>40
Pyrazinamide	—	24	22	>40
Para-aminosalicylic acid	—	21	24	27
Infected controls	21	—	—	—

TABLE 3

ACTIVITY OF S-ETHYL-L-CYSTEINE AGAINST STRAINS OF MYCOBACTERIUM TUBERCULOSIS, HUMAN-TYPE, RESISTANT TO PARA-AMINOSALICYLIC ACID, ISONIAZID, AND DIHYDROSTREPTOMYCIN

Average Lung Lesion Score

Strain	Compound for Treatment				Untreated Infected Controls
	S-Ethyl-L-Cysteine Per Cent Concentration in Diet 0.2	DHSM Mg. per Mouse per Day 2.0	PAS Per Cent Concentration in Diet 0.5	INH Per Cent Concentration in Diet 0.05	
Susceptible to DHSM, PAS, INH, and PZA*	0.2	0.0	2.4	0.0	4.0
Resistant to DHSM	1.6	4.0	—	—	4.0
Resistant to PAS	0.2	—	3.9	—	4.0
Resistant to INH	0.2	—	—	4.0	4.0

* DHSM—Dihydrostreptomycin; PAS—Para-aminosalicylic acid; INH—Isoniazid; PZA—Pyrazinamide.

TABLE 4

ACTIVITY OF S-ETHYL-L-CYSTEINE ADMINISTERED SUBCUTANEOUSLY

Mg. per Mouse per Day	Average Lung Lesion Score	
	S-Ethyl-L-Cysteine	Dihydrostreptomycin
5.0	2.2	—
2.5	3.3	0.0
1.25	3.9	0.2
0.625	4.0	2.7
Infected control 0.0	4.0	

survival time was used as the criterion of efficacy; the findings of an evaluation by this method are summarized in table 2.

S-Ethyl-L-cysteine was effective against strains of tubercle bacilli which were resistant to dihydrostreptomycin, para-aminosalicylic acid, or isoniazid. The pertinent data are given in table 3. These data indicate that S-ethyl-L-cysteine showed a comparable degree of activity against strains resistant to known antituberculous drugs as against a strain which was susceptible to these agents. The average lung lesion score of 1.6 for mice infected with the dihydrostreptomycin-resistant strain and treated with S-ethyl-L-cysteine approaches the upper limit of normal variations and is not a significant difference. The averaged gross lung score for a series of thirty-five trials for S-ethyl-L-cysteine, administered at 0.2 per cent concentration in diet, was 0.6 plus-minus 1.0.

S-Ethyl-L-cysteine did not confer a high degree of protection when administered subcutaneously. The results of a trial with dihydrostreptomycin as the positive control are shown in table 4. S-Ethyl-L-cysteine showed moderate activity at 5 mg., doubtful activity at 2.5 mg., and no activity at 1.25 mg. Dihydrostreptomycin was highly active at 1.25 mg. and slightly to moderately active at 0.625 mg.

S-Ethyl-L-cysteine did not show *in vitro* activity against strains of human, bovine, and avian type in concentrations as high as 100 γ per ml. either in the presence or absence of serum.

SUMMARY AND CONCLUSION

S-Ethyl-L-cysteine is active against experimental tuberculosis in the mouse. When administered as a component of diet, it is more active than para-aminosalicylic acid and pyrazinamide, but less active than streptomycin or isoniazid. It shows a low degree of activity when administered subcutaneously. Because of the absence of *in vitro* activity, the rate at which resistance of the tubercle bacillus develops toward this and related compounds can be determined only by therapeutic trial.

SUMARIO

La S-Etil-L-Cisteína, Miembro de un Nuevo Grupo de Compuestos Antituberculosos

La S-Etil-L-Cisteína es activa contra la tuberculosis experimental en el ratón. Cuando se administra como componente de la alimentación, resulta más activa que el ácido para-aminosalicílico y la piracinamida, pero menos que la estreptomicina o la isoniacida. Por vía subcutánea, revela poca actividad. Debido a la falta de actividad *in vitro*, no puede determinarse más que por medio de pruebas terapéuticas la velocidad con que se forma la resistencia del bacilo tuberculoso a este compuesto y sus afines.

RESUME

S-Éthyl-L-cystéine, l'un des membres du nouveau groupe de composés antituberculeux

La S-éthyl-L-cystéine est active contre la tuberculose expérimentale de la souris. Quand elle est administrée comme constituant du régime alimentaire, son activité est supérieure à celle l'acide para-aminosalicylique et de la pyrazinamide, mais inférieure à celle de la streptomycine ou de l'isoniazide.

Elle possède un faible degré d'activité quand elle est administrée par voie sous-cutanée. En raison de l'absence d'activité *in vitro*, la vitesse à laquelle se produit la résistance du B.K. au composé, ou composés apparentés, ne peut être déterminée que par l'essai thérapeutique.

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RE-TREATMENT OF ADVANCED PULMONARY TUBERCULOSIS WITH VIOMYCIN[1,2]

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INTRODUCTION

Following the reports in 1951 (1–8) that viomycin was effective against *Mycobacterium tuberculosis in vitro* and in experimental animals and could be administered to human subjects (9, 10), a study was initiated in Veterans Administration hospitals to further explore the activity of the antimicrobial in certain types of cases of pulmonary tuberculosis. Preliminary reports (9, 10) had indicated that, with daily dosages of 30 to 75 mg. of viomycin per kg. of body weight, there was considerable toxicity (albuminuria, cylindruria, electrolytic disturbances, impairment of vestibular function, deafness, and some allergic manifestations). Additional clinical investigations have since been reported (11–16). There is general agreement that viomycin is effective in the treatment of tuberculosis, although significantly less so than streptomycin, but that daily dosages in the range of 40 to 60 mg. per kg. of body weight are too toxic for most persons. Dosages of the same range given twice a week instead of daily, however, are associated with much less toxicity and permit clinical use of viomycin in the treatment of tuberculosis when a need arises for an antimicrobial other than those commonly employed.

At the Eleventh Conference of the U. S. Veterans Administration, Army, and Navy on the Chemotherapy of Tuberculosis, in January, 1952, it was decided (17) to initiate a cooperative study of the antituberculous effect of viomycin. The patients to be chosen for treatment were to be those with bacteriologically proved pulmonary tuberculosis who had received considerable chemotherapy and who were discharging tubercle bacilli. The study was organized in hospitals equipped to perform the rather extensive laboratory testing necessary to control toxicity and to study the emergence of viomycin-resistant tubercle bacilli. At the discretion of the individual investigators, one of the following three regimens could be used without random selection:

1. Viomycin alone, 2 gm. a day twice a week (equivalent to approximately 30 to 35 mg. per kg. of body weight)
2. Viomycin, in the same dosage, plus para-aminosalicylic acid (PAS), 12 gm. a day
3. Viomycin, in the same dosage, plus 1 gm. of streptomycin twice a week, both drugs to be administered on the same day

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[1] From the Tuberculosis Service, Veterans Administration Hospital; and the Department of Medicine, University of Minnesota, Minneapolis 14, Minnesota.

[2] Results were compiled by the author on behalf of the staffs of the following Veterans Administration Hospitals, where the study was carried out: Atlanta, Ga. (formerly Chamblee, Ga.); Baltimore, Md.; Brooklyn, N. Y.; Castle Point, N. Y.; Dayton, Ohio; East Orange, N. J.; Hines, Ill.; Houston, Tex., Long Beach, Calif.; Madison, Wis.; McKinney, Tex.; Memphis (Crump Boulevard Hospital and Kennedy Medical Teaching Group Hospital), Tenn.; Minneapolis, Minn.; Richmond, Va.; and San Fernando, Calif.

[3] Present address: Veterans Administration Hospital, Durham, North Carolina.

Two Veterans Administration hospitals had participated in the preliminary trials with daily viomycin which were reported in January, 1952 (18, 19). Ten other Veterans Administration hospitals began treating suitably selected patients in February, 1952. Preliminary reports on these were available in October, 1952 (20), and in February, 1953 (21), in a total of 70 patients, treated from two to six months. The conclusions reached at this time were that:

1. Viomycin is a valuable adjunct in the treatment of tuberculosis.
2. It is most effective when used in combination with other antimicrobial agents.
3. Because of its potential toxicity, even on a twice-a-week regimen, viomycin should not be used when other antimicrobial agents are available, and its use should be restricted to specific treatment problems, chiefly those arising from the failure of prior antimicrobial therapy.

The original study in the Veterans Administration was continued in most of the ten hospitals but was gradually extended to other Veterans Administration hospitals, and other combinations of antimicrobial therapy were investigated. The present report represents a compilation and analysis of 125 patients treated in sixteen hospitals, with follow-up observations available through March, 1954. The great majority of the patients included in the series had far advanced cavitary pulmonary tuberculosis which was progressive and infectious prior to the start of viomycin therapy and which represented the failure of a great deal of prior antimicrobial therapy.

METHODS

A standardized method of recording data on patients with pulmonary tuberculosis treated with antimicrobials has been developed in the cooperative group of Veterans Administration hospitals (22, 23). Observations made on the patients treated with viomycin[4] were made in this manner and were reported to the compiler on individual case report forms, permitting maximum objectivity and flexibility of analysis (24).

The protocol for the treatment of patients with viomycin (17) required the following routine laboratory tests before treatment was started and during viomycin therapy:

A routine analysis once a week
A phenolsulfonthalein (PSP) test for renal function every two weeks (fifteen- and thirty-minute specimens)
An endogenous creatinine clearance every two weeks after the appearance of urinary abnormalities
Serum determinations of sodium, potassium, chlorides, and carbon dioxide-combining power weekly, or more frequently as indicated
An electrocardiogram every six weeks
A bromsulfalein (BSP) liver function test monthly (45-minute sample)
Hemoglobin determination, leukocyte count, and differential blood count weekly
An audiogram monthly (supplemented by tuning fork tests weekly)
A caloric stimulation test monthly

[4] Viomycin was kindly supplied for the greater part of this investigation through the courtesy of Dr. Gladys L. Hobby, Chas. Pfizer & Co., Inc., 630 Flushing Avenue, Brooklyn, N. Y.

Culture of sputum and/or gastric lavage specimen every two weeks and *in vitro* tests of susceptibility to streptomycin and viomycin (in tubes containing 0, 10, 50, and 100γ per ml.) of all strains of tubercle bacilli obtained

Standard 14- by 17-inch chest roentgenograms every month, with tomograms as indicated

Methods of analysis: The results have been observed at the following intervals after the start of viomycin therapy. To the extent to which they were available (four months, eight months, twelve months, eighteen months, and twenty-four months), the results have been tabulated and reported at these intervals. They are usually expressed as the percentage of patients on whom data were available for a given period. In the case of survival the life-table method of analysis has been employed.

Because the number of patients treated on certain of the regimens was small (table 1), in most of the tables there has been a grouping of regimens, in addition to tabulation of the results by individual regimens separately. The three principal grouped regimens are as follows:

Viomycin-PAS-others[5] includes viomycin and PAS; viomycin, isoniazid, and PAS; viomycin, streptomycin, and PAS; viomycin, isoniazid, streptomycin, and PAS.

Viomycin-streptomycin-others includes viomycin and streptomycin; viomycin, isoniazid, and streptomycin; viomycin, streptomycin, and PAS; viomycin, isoniazid, streptomycin, and PAS.

Viomycin-isoniazid-others includes viomycin and isoniazid; viomycin, isoniazid, and streptomycin; viomycin, isoniazid, and PAS; viomycin, isoniazid, streptomycin, and PAS.

As will be noted, the background characteristics of the patients treated on these groups of regimens were very similar. The method of presentation also permitted inspection of results on the individual regimens comprising the groups. A fourth group, composed of viomycin, oxytetracycline, and pyrazinamide, is also included in the tables. Percentages have not been calculated when the number of patients in a treatment group was less than 12 at a given point. The significance of the differences has not been calculated.

Clinical Material

Regimens: In table 1 are summarized the number and the distribution of the patients treated in the sixteen hospitals and the type of therapy administered. The three largest numbers of patients who received uniform therapy were treated with: (*a*) viomycin alone, 31 patients; (*b*) viomycin-PAS, 27 patients; and (*c*) viomycin-isoniazid, 30 patients. Thirty-seven additional patients were treated on seven other regimens for a total of 125 patients. Reports were requested from and submitted by participating hospitals only on patients who had completed at least four months of viomycin therapy or whose viomycin therapy had been terminated prior to the four-month point either by death or by toxic manifestations which necessitated discontinuation of therapy.

Age, race, sex: The age range of patients treated was 18 to 63 years, with a mean of 40.45 years, and there was a slightly bimodal distribution roughly corresponding to the veteran population of the participating hospitals except that there are relatively fewer younger

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[5] Whenever employed in this report, the following antimicrobials were given in the following standard dosages:

Viomycin—1 gm. twice daily twice a week
Streptomycin—1 gm. twice a week
Isoniazid—300 mg. a day
PAS—12 gm. a day
Oxytetracycline (Terramycin®)—2 gm. a day
Pyrazinamide (Aldinamide®)—3 gm. a day

TABLE 1

NUMBER OF PATIENTS TREATED WITH VIOMYCIN

By Hospital and Type of Regimen

Hospital	Regimens*										All Regimens
	VM Alone	VM-PAS	VM-INH	VM-SM	VM-INH-SM	VM-INH-PAS	VM-SM-PAS	VM-INH-SM-PAS	VM-OTC	VM-PZA	
Atlanta	1			2					1		4
Baltimore	3		2					1			6
Brooklyn	3	1	1								5
Castle Point			2								2
Dayton		6	6	2		1		1	6	1	23
East Orange								2			2
Hines	5	3									8
Houston	2										2
Long Beach	5	6			1			2			14
Madison		1	7						1		9
McKinney	2			1			2	1			6
Memphis (Crump)		1	1								2
Memphis (Kennedy)	4	6	6		2				1	1	20
Minneapolis	2	2	3			5					12
Richmond	3	1	1						2		7
San Fernando	1		1					1			3
Total	31	27	30	5	3	6	2	8	11	2	125

* Symbols stand for the following antimicrobials, given in the following standard dosages:

VM—viomycin, 1 gm. twice daily twice a week
SM—streptomycin, 1 gm. twice a week
INH—isoniazid, 300 mg. a day
PAS—para-aminosalicylic acid, 12 gm. a day
OTC—oxytetracycline, 2 gm. a day
PZA—pyrazinamide, 3 gm. a day

patients in the series. There were 97 white patients in the series (77.6 per cent), 24 nonwhites (19.2 per cent), and 4 in whom the color was not stated (3.2 per cent). All patients with available data on sex (121) were males.

Prior antituberculous antimicrobial therapy: The duration of antituberculous drug therapy which the 125 patients had received prior to the start of viomycin therapy is summarized in table 2. In this table, the terms "multiple-drug therapy" and "single-drug therapy" are used irrespective of the drugs employed. In the majority of cases, the single-drug therapy had been streptomycin or PAS and the multiple-drug therapy had been streptomycin, PAS, and isoniazid in various pairings or as a trio. Thirty-four patients (27.2 per cent) had received no prior *single*-drug therapy, 11 (8.8 per cent) no prior *multiple*-drug therapy, but only 3 patients (2.4 per cent) had received *no* prior antimicrobial therapy (one of these was subsequently treated with viomycin alone, one with viomycin-PAS, and one with viomycin-streptomycin-PAS). The average duration of prior single-drug therapy was 5.7 months, and of multiple-drug therapy was 11.7 months, for an average total duration of prior therapy of 17.4 months.

Other characteristics: Other characteristics of the patients treated with the various viomycin regimens are summarized in table 3. The strains of tubercle bacilli obtained

TABLE 2

DURATION OF PRIOR ANTITUBERCULOUS ANTIMICROBIAL THERAPY

Months of Prior Therapy	Viomycin Regimens*										All Regimens	Per Cent of Total
	VM Alone	VM-PAS	VM-INH	VM-SM	VM-INH-SM	VM-INH PAS	VM-SM PAS	VM INH-SM PAS	VM OTC	VM PZA		
Multiple-drug therapy												
None	3	4	2				1		1		11	8.8
1–2	3	1	1	2							7	5.6
3–4	3	1	2	1			1	1	2		11	8.8
5–6	2	4	1			1		1			9	7.2
7–9	3	5	4		3	1		1	1		18	14.4
10–12	4	2	6					2	1		15	12.0
13–15	4	3	2					1	1	2	13	10.4
16–18	3	1	4					1	1		10	8.0
19–21	3	2	3			1		1	2		12	9.6
22–24		2	2								4	3.2
25–30	1	1	1			2					5	4.0
31–36		1	2			1					4	3.2
37–38	1								1		2	1.6
Unknown	1			2					1		4	2.4
Totals	31	27	30	5	3	6	2	8	11	2	125	100.0
Mean (months)	10.7	10.9	13.5	2.3	7.3	21.2	1.5	11.0	13.6	13.5	11.7	—
Single-drug therapy												
None	9	10	5			1	2	4	3		34	27.2
1–2	7	1	2			2				1	13	10.4
3–4	7	3	9		2			1	1		23	18.4
5–6	3		2		1	1			1		8	6.4
7–9	2	6	3	2		1		1	2	1	18	14.4
10–12		4	4					1	1		10	8.0
13–15	1	2	3						1		7	5.6
16–18											0	0.0
19–24	1					1		1	1		4	3.2
25–30		1	2								3	2.4
31–33				1							1	0.8
Unknown	1			2					1		4	3.2
Totals	31	27	30	5	3	6	2	8	11	2	125	100.0
Mean (months)	3.6	6.0	6.8	16.3	4.3	5.8	0.0	5.8	6.8	4.5	5.7	—
Average total duration of prior therapy, multiple and single (in months)	14.3	16.9	20.3	18.6	11.6	27.0	1.5	16.8	20.4	18.0	17.4	—

* *See* footnote to table 1.

from 83 of 109 patients on whom the data were available (76 per cent) showed complete *in vitro* resistance to 10γ of streptomycin per ml.; and in 46 of 104 patients (44 per) cent, the strains were resistant to 100γ of streptomycin per ml. In only 7 of 109 patient-strains (6.5 per cent) was there no growth of the tubercle bacilli in 10γ per ml., and in 27 of 104 patient-strains (26 per cent) there was no growth in 100γ of streptomycin per ml.

TABLE 3

Characteristics of Patients Treated with Viomycin and Other Drugs

At Start of Therapy

Regimens†	*In Vitro* SM Susceptibility						Bacteriology						Stage, Far Advanced Disease			Pretreatment Roentgenogram Trend Progressive			Cavitary Status					
	Completely Resistant to 10 γ			Completely Resistant to 100 γ			Sputum Positive on Smear or Concentrate			Sputum Gastric Washings Positive on Culture									Multiple Cavities			Cavities More Than 4 Cm. in Diameter		
	Observed	Number	Per cent	Observed	Number	Per cent	Observed	Number	Per cent	Observed	Number	Per cent	Observed	Number	Per cent	Observed	Number	Per cent	Observed	Number	Per cent	Observed	Number	Per cent
VM alone	29	25	86	27	13	48	31	29	94	31	30	97	31	29	94	30	18	60	30	24	80	25	18	72
VM-PAS	25	20	80	19	8	42	27	24	89	27	25	92	27	26	96	27	18	67	27	14	52	27	14	52
VM-INH	24	17	71	24	11	46	30	30	100	30	30	100	30	28	93	30	12	40	27	15	56	27	13	48
VM-SM	5	4	*	5	1	*	5	4	*	5	4	*	5	4	*	3	2	*	3	3	*	2	1	*
VM-INH-SM	3	3	*	3	3	*	3	3	*	3	3	*	3	3	*	3	3	*	3	2	*	3	3	*
VM-INH-PAS	6	3	*	6	2	*	6	3	*	6	3	*	6	5	*	6	1	*	6	3	*	5	2	*
VM-SM-PAS	2	0	*	2	0	*	2	2	*	2	2	*	2	2	*	2	1	*	2	1	*	2	2	*
VM-INH-SM-PAS	6	3	*	6	3	*	8	7	*	8	7	*	8	8	*	8	4	*	8	3	*	8	2	*
VM-OTC	10	7	*	10	4	*	11	10	*	11	10	*	11	11	*	10	7	*	9	3	*	9	4	*
VM-PZA	2	1	*	2	0	*	2	2	*	2	2	*	2	2	*	2	1	*	2	2	*	2	2	*
Grouped regimens																								
VM-PAS-others	41	26	63	33	13	39	43	36	84	43	37	86	43	41	95	43	24	56	43	21	49	42	20	48
VM-SM-others	16	10	63	16	7	44	18	16	88	18	16	88	18	17	94	16	10	63	16	9	56	15	8	53
VM-INH-others	39	26	67	39	19	49	47	43	91	47	43	91	47	44	94	47	20	43	44	23	52	43	20	47
VM-OTC and VM-PZA	12	8	67	12	4	33	13	12	92	13	12	92	13	13	100	12	8	67	11	5	46	11	6	56
All regimens	109	83	76	104	46	44	125	115	92	125	117	94	125	119	95	121	68	56	117	70	60	108	61	57

* Percentage not calculated when number of cases in group is less than 12.

† *See* footnote to table 1.

All patients had been discharging tubercle bacilli prior to the start of viomycin therapy. In 115 patients (92 per cent), the bacilli had been demonstrated by microscopy and in sputum, in 117 (94 per cent) by culture of sputum or gastric lavage specimens, at the start of viomycin therapy. In only 6 patients (4.8 per cent) were tubercle bacilli not demonstrated by microscopy or culture at the time of starting viomycin therapy. One of the patients was later treated with viomycin alone; one with viomycin–streptomycin; 2 with viomycin-isoniazid–PAS; one with viomycin–isoniazid–streptomycin–PAS; and one with viomycin–oxytetracycline.

One hundred and nineteen patients (95 per cent) had far advanced pulmonary tuberculosis at the start of viomycin therapy; the remainder had moderately advanced disease. Among 121 patients on whom the information was reported, 68 (56 per cent) had tuberculous disease which was progressive in the period immediately prior to viomycin therapy, 45 (37 per cent) had stationary disease, and in 8 (7 per cent) it was regressive.

Cavitary status was reported at the start of viomycin therapy in 117 patients. In 19 (16 per cent) there was no detectable cavitation, in 28 (24 per cent) there were single cavities, and in 70 (60 per cent) there were multiple cavities. The diameter of the largest cavity was estimated in 108 patients. In 19 (18 per cent), no cavity was detectable; in 15 (14 per cent), it was 2 cm. or less; in 13 (12 per cent), between 2.5 and 3.5 cm.; in 45 (42 per cent), 4.0 to 7.5 cm.; and in 16 (15 per cent), 8 cm. or more in diameter.

Duration of Antimicrobial Therapy

Duration of viomycin therapy: The duration of antimicrobial therapy which patients received on the various viomycin regimens is summarized in table 4. The average for the

TABLE 4

DURATION OF THERAPY WITH VIOMYCIN

According to Regimen

Regimen*	Duration in Months						Total Patients	Average
	1–3	4–6	7–9	10–12	13–15	16–18		
VM alone	8	19	4				31	4.5
VM-PAS	2	18	4		1	2	27	6.0
VM-INH	7	12	7	4			30	5.9
VM-SM	1	3	1				5	4.6
VM-INH-SM		1	2				3	7.3
VM-INH-PAS		2	3	1			6	8.0
VM-SM-PAS			1		1		2	11.0
VM-INH-SM-PAS		4	4				8	6.1
VM-OTC	3	6		1		1	11	5.9
VM-PZA	1	1					2	2.5
Grouped regimens								
VM-PAS-others	2	24	12	1	2	2	43	6.0
VM-SM-others	1	8	8		1		18	6.4
VM-INH-others	7	19	16	5			47	6.3
VM-OTC and VM-PZA	4	7		1		1	13	5.3
All regimens	22†	66	26	6	2	3	125	5.7
Per cent of total	17.6	52.8	20.8	4.8	1.6	2.4	100.0	—

* *See* footnote to table 1.

† Therapy discontinued in less than 4 months for following reasons: died, 5; toxicity, 11; spreads, 6.

TABLE 5

TERMINATION OF VIOMYCIN THERAPY

Regimen*	Medical Reasons				Administrative Reasons		Completed Planned Course	Subtotal Therapy Terminated	Continuing Therapy at Time of Report	Total
	Died	VM Toxicity	Spread	VM Resistance	Irregular Discharge	Supply Temporarily Exhausted				
VM alone	2	5	3		1	1	16	28	3	31
VM-PAS	1	3	1			1	17	23	4	27
VM-INH	3	5	1				7	16	14	30
VM-SM	2						3	5	0	5
VM-INH-SM		1		1			0	2	1	3
VM-INH-PAS							2	2	4	6
VM-SM-PAS	1						1	2	0	2
VM-INH-SM-PAS		1			1		0	2	6	8
VM-OTC	2	3	1	1			0	7	4	11
VM-PZA			1				0	1	1	2
Grouped regimens										
VM-PAS-others	2	4	1		1	1	20	29	14	43
VM-SM-others	3	2		1	1		4	11	7	18
VM-INH-others	3	7	1	1	1		9	22	25	47
VM-OTC and VM-PZA	2	3	2	1			0	8	5	13
All regimens	11	18	7	2	2	2	46	88	37	125
Per cent of 125 patients	8.8	14.4	5.6	1.6	1.6	1.6	36.8	70.4	29.6	100.0
Per cent of 88 patients with therapy terminated	12.5	20.4	7.9	2.3	2.3	2.3	52.3	100.0	—	—

* *See* footnote to table 1.

entire group of 125 patients was 5.7 months. In general, the duration was less for the group treated with viomycin alone than on the regimens of viomycin combined with other drugs. Twenty-two patients in this series (17.6 per cent) had less than 4 months of therapy. The viomycin had been discontinued for the following reasons: died, 5; viomycin toxicity, 11; spread of the tuberculous disease, 6. In a total of 88 patients (70.4 per cent), the duration of therapy was 6 months or less. The longest period of treatment was 18 months.

Termination of viomycin therapy: In table 5 it will be seen that, at the time of reporting, 37 patients (29.6 per cent) were still receiving viomycin. Of the remaining 88 patients in whom the therapy had been terminated (70.4 per cent of the total), in 38 (43.1 per cent) it had been discontinued for medical reasons (died during therapy, 11; viomycin toxicity, 18; spread of disease, 7; emergence of viomycin resistance, 2); for administrative reasons (premature irregular discharge and temporary exhaustion of drug supply) in 4 (4.6 per cent); and the planned course was completed in 46 (52.3 per cent). The lowest rate of discontinuation for medical reasons was in the group treated with viomycin–PAS and other drugs; the highest in the group treated with viomycin–oxytetracycline–pyrazinamide.

Additional antimicrobial therapy: Following the completion of viomycin therapy, whether planned or premature, there were indications for additional antimicrobial therapy in a considerable number of patients in the series, as indicated in table 6. Of the 66 patients whose viomycin therapy was terminated and on whom the status of additional antimicrobial

TABLE 6

ADDITIONAL ANTIMICROBIAL THERAPY AFTER TERMINATION OF VIOMYCIN THERAPY

Regimen†	Number of Patients	Additional Therapy Not Reported	Therapy Continuing	Therapy Terminated (Death)	Alive after VM Therapy Additional Status Known	Received Additional Therapy		Received No Additional Therapy		Average Number of Months of Additional Chemotherapy
						Number	Per Cent	Number	Per Cent	
VM alone	31	2	3	3	23	3	13	20	87	12.3
VM-PAS	27	1	4	1	21	3	14	18	86	9.8
VM-INH	30	2	14	3	11	5	44	6	56	6.7
VM-SM	5	2		2	1	0	*	1	*	7.0
VM-INH-SM	3		1		2	0	*	2	*	4.5
VM-INH-PAS	6		4		2	1	*	1	*	3.0
VM-SM-PAS	2	1		1	0	0	*	0	*	0.0
VM-INH-SM-PAS	8	1	6		1	0	*	1	*	1.0
VM-OTC	11	1	4	2	4	0	*	4	*	15.5
VM-PZA	2		1		1	0	*	1	*	15.0
Grouped regimens										
VM-PAS-others	43	3	14	2	24	4	17	20	83	9.1
VM-SM-others	18	4	7	3	4	0	*	4	*	4.3
VM-INH-others	47	3	25	3	16	6	37	10	63	5.3
VM-OTC and VM-PZA	13	1	5	2	5	0	*	5	*	15.4
All regimens	125	10	37	12‡	66	12	18	54	82	10.4
Per cent of total	100.0	8.0	29.6	9.6	52.8	9.6	—	43.2	—	—

* Percentage not calculated when number of patients in group is less than 12.

† *See* footnote to table 1.

‡ One patient died two days after terminating VM therapy—*see* table 5.

therapy was known, 54 (82 per cent) received additional drug therapy, averaging 10.4 months per patient.

RESULTS

Viomycin Toxicity

In table 7 are summarized the incidence of various manifestations of toxicity to viomycin and (in parentheses) the number of instances in which it was believed necessary to discontinue viomycin toxicity because of such toxic manifestations. Because some manifestations occurred more than singly in the same patient, their incidence is also given by number of patients (eliminating multiple manifestations) in the next to last column.

Treatment was stopped because of toxicity in 18 patients (14.4 per cent), with 24 toxic manifestations. The individual manifestations and the frequency of their occurrence were: impairment of renal function, 3 instances; severe albuminuria, 1; electrolytic disturbance, 2; fever, 2; angioneurotic edema, 2; drug rash, 3; impairment of auditory function, 2; impairment of vestibular function of significant degree, 2; serious nausea and vomiting, 5; significant decrease in hemoglobin, 1;

TABLE 7

MANIFESTATIONS OF TOXICITY DURING VIOMYCIN THERAPY

Regimen*	Number Patients in Group	Renal Allergic						Eighth Cranial Nerve		Other				Total Toxic Manifestations	Total Patients with Toxic Manifestations	Total Patients with "Serious" Toxicity
		Renal Function Impairment	Albuminuria	Electrolytic Disturbance	Fever	Angioneurotic Edema	Rash	Auditory	Vestibular	Nausea and Vomiting	Hemoglobin Decreased	Eosinophilia	Severe Pain at Injection Site			
VM alone	31	4 (3)†	3	3 (1)	3 (1)				1 (1)	1 (1)		6	1 (1)	22 (8)	18 (5)	9
VM-PAS	27		2		1 (1)		1 (1)			1 (1)	1 (1)	4		10 (4)	7 (3)	3
VM-INH	30					2 (2)	2 (2)			1 (1)		1		6 (5)	6 (5)	5
VM-SM	5	2										2		4	2	2
VM-INH-SM	3								1 (1)	1 (1)				2 (2)	1 (1)	1
VM-INH-PAS	6										1			1	1	0
VM-SM-PAS	2													0	0	0
VM-INH-SM-PAS	8		1 (1)											1 (1)	1 (1)	1
VM-OTC	11	1		1 (1)				2 (2)		1 (1)		2		7 (4)	5 (3)	4
VM-PZA	2													0	0	0
Grouped regimens																
VM-PAS-others	43		3 (1)		1 (1)		1 (1)			1 (1)	2 (1)	4		12 (5)	9 (4)	4
VM-SM-others	18	2	1 (1)						1 (1)			2		7 (3)	4 (2)	4
VM-INH-others	47		1 (1)			2 (2)	2 (2)		1 (1)	2 (2)	1	1		10 (8)	9 (7)	7
VM-OTC and VM-PZA	13	1		1 (1)				2 (2)		1 (1)		2		7 (4)	5 (3)	4
Total	125	7 (3)	6 (1)	4 (2)	4 (2)	2 (2)	3 (3)	2 (2)	2 (2)	5 (5)	2 (1)	15	1 (1)	53 (24)	41 (18)	25
Per cent of total																
All toxicity	100.0	5.6	4.2	3.2	3.2	1.6	2.4	1.6	1.6	4.0	1.6	12.0	0.8	42.4	32.8	—
Therapy stopped because of toxicity		2.4	0.8	1.6	1.6	1.6	2.4	1.6	1.6	4.0	0.8		0.8	19.2	14.4	20.0

* *See* footnote to table 1.

† Numbers in parentheses = number of patients with therapy discontinued because of toxicity.

and severe pain at site of injection, 1 instance. No significant change in electrocardiographic pattern was noted.

The incidence of total toxic manifestations was 53 (42 per cent) in 41 patients (33 per cent); and the toxicity was thought to be "serious" in 25 patients (20 per cent), the 18 in whom therapy was discontinued and 7 others.

"Serious" toxicity was slightly higher in the patients treated with streptomycin (4 of 18 patients, or 22 per cent) than in the other grouped regimens, but was highest in the patients treated with viomycin alone (9 of 31, or 29 per cent) and with viomycin–oxytetracycline–pyrazinamide (4 of 13, or 31 per cent). Combined administration of viomycin and streptomycin apparently did not significantly increase the impairment of function of either branch of the eighth cranial nerve although, in prospect, there had been apprehension in this regard.

Roentgenographic Observations

Evaluations of roentgenographic change in the cooperative study in the Veterans Administration, Army, and Navy have been made on a seven-point scale; films obtained at stated intervals are compared with the film taken immediately prior to the initiation of antimicrobial therapy (22). The results according to this type of evaluation are given in table 8, expressed in numbers and percentages of patients who showed all degrees of improvement (slight, moderate, and marked) and those who showed moderate plus marked improvement.

In general there was an increase in the percentage of improvement by either criterion with increased duration of observation; the improvement of moderate plus marked degree increased more slowly. There was definitely less improvement by either criterion among the patients treated with viomycin alone than among those who received viomycin with other antimicrobials. Viomycin with oxytetracycline (and pyrazinamide) also appeared to result in lower rates of improvement; but among the other three grouped regimens (viomycin-PAS and others, viomycin-streptomycin and others, viomycin-isoniazid and others), the rates appeared to be approximately equivalent.

Cavity closure: Investigators were asked to report on the individual case report form whether cavities previously identified had been "closed" or "lost to view" at each report period, as determined by roentgenography, including tomography when indicated. The rates of closure of cavities are given in table 9. The results rather closely paralleled the trends reported for roentgenographic improvement. The rates of cavity closure were higher with multiple-drug therapy except for patients treated with viomycin alone or with oxytetracycline or pyrazinamide along with viomycin.

Roentgenographic relapse: Serial roentgenographic observation also permitted a reporting of the incidence of "unfavorable roentgenographic change" after a prior period of absence of such change. Thus defined, there were 61 "roentgenographic relapses" in 39 patients (31.2 per cent of the total group). In table 10 the incidence of "roentgenographic relapse" is given according to viomycin regimen and duration of observation. Roentgenographic relapses subsequent to the first are not reflected in this table. The relapse rates increased with duration

ROENTGENOGRAPHIC IMPROVEMENT WITH VIOMYCIN THERAPY

Regimen†	Duration of Observation														
	1–4 months			5–8 months			9–12 months			13–18 months			19–24 months		
	Number of Patients Observed	All Degrees of Improvement	Moderate and Marked Improvement	Number of Patients Observed	All Degrees of Improvement	Moderate and Marked Improvement	Number of Patients Observed	All Degrees of Improvement	Moderate and Marked Improvement	Number of Patients Observed	All Degrees of Improvement	Moderate and Marked Improvement	Number of Patients Observed	All Degrees of Improvement	Moderate and Marked Improvement
VM alone	30	6	1	27	7	2	20	6	1	16	5	1	13	5	1
(per cent improved)		(20)	(3)		(26)	(7)		(30)	(5)		(31)	(6)		(38)	(8)
VM-PAS	27	11	3	23	13	3	18	12	5	17	13	6	10	9	4
(per cent improved)		(41)	(11)		(56)	(13)		(67)	(28)		(76)	(35)		*	*
VM-INH	30	13	3	22	11	3	12	8	4	4	4	2	—	—	—
(per cent improved)		(43)	(10)		(50)	(14)		(67)	(33)		*	*		—	—
VM-SM	5	1	0	4	1	1	2	0	0	—	—	—	—	—	—
VM-INH-SM	3	0	0	3	1	0	2	1	0	—	—	—	—	—	—
VM-INH-PAS	6	2	0	6	4	0	3	2	0	—	—	—	—	—	—
VM-SM-PAS	2	1	0	2	1	1	2	1	1	2	1	1	—	—	—
VM-INH-SM-PAS	8	7	1	7	6	3	1	1	0	—	—	—	—	—	—
VM-OTC	11	6	0	8	4	1	6	4	1	4	2	1	3	0	0
VM-PZA	2	1	0	1	0	0	1	1	0	1	0	0	—	—	—
Grouped regimens															
VM-PAS-others	43	21	4	38	24	7	24	16	6	19	14	7	10	9	4
(per cent improved)		(49)	(9)		(63)	(18)		(67)	(25)		(74)	(38)		*	*
VM-SM-others	18	9	1	16	9	5	7	3	1	2	1	1	—	—	—
(per cent improved)		(50)	(6)		(56)	(31)		*	*		*	*		—	—
VM-INH-others	47	22	4	38	22	6	18	12	4	—	—	—	—	—	—
(per cent improved)		(47)	(9)		(58)	(16)		(67)	(22)		—	—		—	—
VM-OTC and VM-PZA	13	7	0	9	4	1	7	5	1	5	2	1	3	0	0
(per cent improved)		(54)	(0)		*	*		*	*		*	*		*	*
All regimens	124	48	8	103	48	14	67	36	12	44	25	11	26	14	5
(per cent improved)		(39)	(6)		(47)	(14)		(54)	(18)		(57)	(25)		(54)	(19)

* Percentage not calculated when number of cases in group is less than 12.

† *See* footnote to table 1.

TABLE 9

CAVITY CLOSURE DURING AND AFTER VIOMYCIN THERAPY

Regimen†	Number Patients in Group	Duration of Observation																	
		1-4 months			5-8 months			9-12 months			13-18 months			19-24 months			All Durations		
		Number with Observations Available	Cavity Closed		Number with Observations Available	Cavity Closed		Number with Observations Available	Cavity Closed		Number with Observations Available	Cavity Closed		Number with Observations Available	Cavity Closed		Number with Observations Available	Cavity Closed	
			Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent
VM alone	31	27	2	7	22	4	18	16	2	13	13	1	8	10	1	*	27	5	19
VM-PAS	27	21	2	10	18	4	22	16	4	25	16	6	38	10	5	*	21	9	43
VM-INH	30	25	3	12	18	7	39	10	3	*	4	1	*	—	—	—	25	8	32
VM-SM	5	3	0	*	2	1	*	2	1	*	—	—	—	—	—	—	3	1	*
VM-INH-SM	3	3	0	*	3	0	*	2	0	*	—	—	—	—	—	—	3	0	*
VM-INH-PAS	6	2	0	*	2	0	*	—	—	—	—	—	—	—	—	—	2	0	*
VM-SM-PAS	2	2	0	*	2	1	*	2	1	*	2	2	*	—	—	—	2	2	*
VM-INH-SM-PAS	8	5	0	*	4	2	*	1	1	*	—	—	—	—	—	—	5	2	*
VM-OTC	11	7	0	*	6	0	*	4	0	*	3	0	*	3	0	*	7	0	*
VM-PZA	2	2	0	*	1	0	*	1	0	*	1	0	*	—	—	—	2	0	*
Grouped regimens																			
VM-PAS-others	43	30	2	7	26	7	27	19	6	32	18	8	44	10	5	*	30	13	43
VM-SM-others	18	13	0	0	11	4	*	7	3	*	2	2	*	—	—	—	13	5	38
VM-INH-others	47	35	3	9	27	9	33	13	4	31	4	1	*	—	—	—	35	10	29
VM-OTC and VM-PZA	13	9	0	*	7	0	*	5	0	*	4	0	*	3	0	*	9	0	*
All regimens	125	97	7	7	78	19	24	54	12	22	39	10	26	23	6	26	97	27	28

* Percentages not calculated when number of patients in group is less than 12.

† *See* footnote to table 1.

TABLE 10

CUMULATIVE ROENTGENOGRAPHIC RELAPSE DURING AND AFTER VIOMYCIN THERAPY

Regimen†	Duration of Observation																	
	1–4 months			5–8 months			9–12 months			13–18 months			19–24 months			All Durations		
	Number of Patients Observed	Relapses		Number of Patients Observed	Relapses		Number of Patients Observed	Relapses		Number of Patients Observed	Relapses		Number of Patients Observed	Relapses		Number of Patients Observed	Relapses	
		Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent
VM alone	31	9	29	27	10	37	20	12	60	16	11	69	13	9	69	31	18	58
VM-PAS	27	3	11	23	4	17	18	4	22	17	4	24	10	2	*	27	5	19
VM-INH	30	5	17	22	6	27	12	6	50	4	1	*	—	—	—	30	8	27
VM-SM	5	1	*	4	1	*	2	1	*	—	—	—	—	—	—	5	1	*
VM-INH-SM	3	1	*	3	1	*	2	1	*	—	—	—	—	—	—	3	1	*
VM-INH-PAS	6	0	*	6	0	*	3	0	*	—	—	—	—	—	—	6	0	*
VM-SM-PAS	2	0	*	2	0	*	2	0	*	2	0	*	—	—	—	2	0	*
VM-INH-SM-PAS	8	0	*	7	0	*	1	0	*	—	—	—	—	—	—	8	0	*
VM-OTC	11	2	*	8	3	*	6	1	*	4	2	*	3	3	*	11	5	*
VM-PZA	2	1	*	1	1	*	1	1	*	1	1	*	—	—	—	2	1	*
Grouped regimens																		
VM-PAS-others	43	3	7	38	4	11	24	4	17	19	4	21	10	2	*	43	5	12
VM-SM-others	18	2	11	16	2	13	7	2	*	2	0	*	—	—	—	18	2	11
VM-INH-others	47	6	13	38	7	18	18	7	39	4	1	*	—	—	—	47	9	19
VM-OTC and VM-PZA	13	3	23	9	4	*	7	2	*	5	3	*	3	3	*	13	6	46
All regimens	125	22	18	103	26	25	67	26	39	44	19	43	26	14	54	125	39	31

* Percentages not calculated when number of patients in group is less than 12.

† *See* footnote to table 1.

TABLE 11

RELAPSES (BY ROENTGENOGRAPHIC OBSERVATION) DURING AND AFTER VIOMYCIN THERAPY

Regimen*	Patients in Group	Patients with Relapses	Total Relapses	Total Months of Observation	Relapses per Month		Relapses per Year	
					per Patient	per Relapse	per Patient	per Relapse
VM alone	31	18	26	446	0.040	0.059	0.48	0.72
VM-PAS	27	5	8	405	0.012	0.020	0.14	0.24
VM-INH	30	8	12	248	0.032	0.048	0.38	0.58
VM-SM	5	1	2	33	0.030	0.061	0.37	0.73
VM-INH-SM	3	1	1	30	0.033	0.033	0.40	0.40
VM-INH-PAS	6	0	0	52	—	—	—	—
VM-SM-PAS	2	0	0	31	—	—	—	—
VM-INH-SM-PAS	8	0	0	55	—	—	—	—
VM-OTC	11	5	9	150	0.033	0.060	0.40	0.72
VM-PZA	2	1	3	20	0.050	0.150	0.60	1.80
Grouped regimens								
VM-PAS-others	43	5	8	543	0.009	0.015	0.11	0.18
VM-SM-others	18	2	3	149	0.013	0.020	0.16	0.24
VM-INH-others	47	9	13	385	0.023	0.034	0.28	0.41
VM-OTC and VM-PZA	13	6	12	170	0.035	0.071	0.42	0.85
All regimens	125	39	61	1,470 (122.5 years)	0.027	0.041	0.32	0.50

* *See* footnote to table 1.

of observation and were highest in the groups treated with viomycin alone and with oxytetracycline or pyrazinamide along with viomycin. The relapse rates for the three "grouped regimens" of PAS, streptomycin, or isoniazid with viomycin were markedly lower (less than 20 per cent) and were at approximately the same level. In table 11 may be seen the incidence of roentgenographic relapse according to total months of observation of patients treated with viomycin, both in terms of relapse per patient (disregarding all relapses after the first) and of total number of relapses. The lowest total relapse rate (0.18 per patient per year of observation) was in the group treated with viomycin–PAS and other drugs; the next higher (0.24 per patient per year) in the group treated with viomycin–streptomycin and other drugs; the median rate (0.41 per patient per year) in the group treated with viomycin–isoniazid and other drugs; the next highest rate (0.72 per patient per year) in the group treated with viomycin alone; and the highest rate (0.85 per patient per year) in the group treated with viomycin and oxytetracycline or pyrazinamide.

As may be seen in table 12, 33 of the 61 relapses (54 per cent) occurred while the patients were still receiving viomycin therapy, in 27 of the 39 patients (69 per cent) who experienced roentgenographic relapse. The total incidence of relapse during viomycin therapy for the entire group was 0.56 per patient per 12 months, with the lowest rate again in the group which received viomycin–PAS and other

TABLE 12

RELAPSES (BY ROENTGENOGRAPHIC OBSERVATION) DURING VIOMYCIN THERAPY ONLY

Regimen*	Patients in Group	Patients with Relapses	Total Relapses	Total Months of Observation	Relapses per Month		Relapses per Year	
					per Patient	per Relapse	per Patient	per Relapse
VM alone	31	11	11	138	0.080	0.080	0.96	0.96
VM-PAS	27	3	4	161	0.019	0.025	0.23	0.30
VM-INH	30	8	11	176	0.045	0.062	0.55	0.75
VM-SM	5	1	2	23	0.043	0.087	0.52	1.04
VM-INH-SM	3	1	1	22	0.045	0.045	0.55	0.55
VM-INH-PAS	6	0	0	48	—	—	—	—
VM-SM-PAS	2	0	0	22	—	—	—	—
VM-INH-SM-PAS	8	0	0	49	—	—	—	—
VM-OTC	11	2	3	65	0.031	0.046	0.37	0.55
VM-PZA	2	1	1	5	0.200	0.200	2.40	2.40
Grouped regimens								
VM-PAS-others	43	3	4	280	0.011	0.014	0.13	0.17
VM-SM-others	18	2	3	116	0.017	0.026	0.20	0.31
VM-INH-others	47	9	13	295	0.030	0.044	0.37	0.53
VM-OTC and VM-PZA	13	3	4	70	0.043	0.057	0.52	0.68
All regimens	125	27	33	709 (59.1 years)	0.038	0.046	0.46	0.56

* *See* footnote to table 1.

drugs. The rates for the other regimens were in approximately the same relative order as for all roentgenographic relapse (above): 0.31 per patient per 12 months for those treated with viomycin–streptomycin and other drugs; 0.53 for those treated with viomycin-isoniazid and other drugs; 0.68 for those treated with viomycin and oxytetracycline or pyrazinamide; and the highest rate, 0.96, for those treated with viomycin alone.

Bacteriologic Observations

Reversal of infectiousness: The presence of tubercle bacilli in sputum or gastric washings is tabulated in table 13 by regimen and time of observation after the start of viomycin therapy. The over-all "conversion" rate was 30 per cent; for the grouped regimens, it was 30 to 50 per cent eight to twelve months after the start of viomycin therapy except among the patients treated with viomycin and oxytetracycline or pyrazinamide (8 per cent), and was approximately half this rate (23 per cent) in the group treated with viomycin alone.

Emergence of viomycin-resistant organisms: The results of *in vitro* tests for viomycin susceptibility (10 and 100 γ of drug per ml.) are given in table 14. The term *complete resistance* is used to designate the appearance of growth equivalent to that in the control tube; *partial resistance* is used to describe growth less than that which occurred in the control tube. The results are presented for each concentra-

TABLE 13

BACTERIOLOGIC STATUS OF PATIENTS TREATED WITH VIOMYCIN

(Conversion of Sputum and/or Gastric Cultures to Negative)

Regimen†	Number of Patients	Duration of Observation in Months																	
		1–4 months			5–8 months			9–12 months			13–18 months			19–24 months			All Durations		
		Number Observed	Converted		Number Observed	Converted		Number Observed	Converted		Number Observed	Converted		Number Observed	Converted		Number Observed	Converted	
			Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent
VM alone	31	31	3	10	27	5	19	20	3	15	16	2	13	13	3	23	31	7	23
VM-PAS	27	27	3	11	23	5	22	18	4	22	17	4	23	10	2	*	27	4	15
VM-INH	30	30	4	13	22	10	45	12	5	42	4	1	*	—	—	—	30	13	43
VM-SM	5	5	1	*	4	2	*	2	0	*	—	—	—	—	—	—	5	2	*
VM-INH-SM	3	3	0	*	3	0	*	2	0	*	—	—	—	—	—	—	3	0	*
VM-INH-PAS	6	6	3	*	6	3	*	3	1	*	—	—	—	—	—	—	6	3	*
VM-SM-PAS	2	2	0	*	2	1	*	2	1	*	2	1	*	—	—	—	2	1	*
VM-INH-SM-PAS	8	8	2	*	7	5	*	1	1	*	—	—	—	—	—	—	8	6	*
VM-OTC	11	11	1	*	8	1	*	6	1	*	4	1	*	3	0	*	11	1	*
VM-PZA	2	2	0	*	1	0	*	1	0	*	1	0	*	—	—	—	2	0	*
Grouped regimens																			
VM-PAS-others	43	43	8	19	38	14	37	24	7	29	19	5	27	10	2	*	43	14	33
VM-SM-others	18	18	3	17	16	8	50	7	2	*	2	1	*	—	—	—	18	9	50
VM-INH-others	47	47	9	19	38	18	48	18	7	39	4	1	*	—	—	—	47	22	46
VM-OTC and VM-PZA	13	13	1	8	9	1	*	7	1	*	5	1	*	3	0	*	13	1	8
All regimens	125	125	17	14	103	32	31	67	16	24	44	11	25	26	5	19	125	37	30

* Percentages not calculated when number of patients in group is less than 12.

† *See* footnote to table 1.

TABLE 14

EMERGENCE OF IN VITRO RESISTANCE TO VIOMYCIN IN PATIENTS TREATED WITH VIOMYCIN ALONE AND IN COMBINATION

Regimen†	Number of Patients	4 Months or Less of VM Therapy					More Than 4 Months of VM Therapy					All Durations VM Therapy				
		Number with Data	Resistant to 10 γ		Resistant to 100 γ		Number with Data	Resistant to 10 γ		Resistant to 100 γ		Number with Data	Resistant to 10 γ		Resistant to 100 γ	
			Complete (Number)	Partial (Number)	Complete (Number)	Partial (Number)		Complete (Number)	Partial (Number)	Complete (Number)	Partial (Number)		Complete (Number)	Partial (Number)	Complete (Number)	Partial (Number)
VM alone	31	8	3	4	2	1	18	11	7	8	2	26	14	11	10	3
(per cent resistant)			*	*	*	*		(61)	(39)	(44)	(11)		(54)	(42)	(39)	(11)
VM-PAS	27	15	5	5	4	0	5	3	2	1	2	20	8	7	5	2
(per cent resistant)			(33)	(33)	(27)	(0)		*	*	*	*		(40)	(35)	(25)	(10)
VM-INH	30	5	1	1	0	0	8	2	3	0	1	13	3	4	0	1
(per cent resistant)			*	*	*	*		*	*	*	*		(23)	(31)	(0)	(8)
VM-SM	5	3	1	2	0	3	2	1	0	0	1	5	2	2	0	4
VM-INH-SM	3	0	0	0	0	0	3	1	2	0	0	3	1	2	0	0
VM-INH-PAS	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VM-SM-PAS	2	0	0	0	0	0	2	0	0	0	0	2	0	0	0	0
VM-INH-SM-PAS	8	0	0	0	0	0	3	2	0	1	1	3	2	0	1	1
VM-OTC	11	6	2	3	1	2	2	0	2	0	0	8	2	5	1	2
VM-PZA	2	2	1	0	0	0	0	0	0	0	0	2	1	0	0	0
Grouped regimens																
VM-PAS-others	43	15	5	5	4	0	10	5	2	2	3	25	10	7	6	3
(per cent resistant)			(33)	(33)	(27)	(0)		*	*	*	*		(40)	(28)	(24)	(12)
VM-SM-others	18	3	1	2	0	3	10	4	2	1	2	13	5	4	1	5
(per cent resistant)			*	*	*	*		*	*	*	*		(38)	(31)	(8)	(38)
VM-INH-others	47	5	1	1	0	0	14	5	5	1	2	19	6	6	1	2
(per cent resistant)			*	*	*	*		(36)	(36)	(7)	(14)		(32)	(32)	(5)	(11)
VM-OTC and VM-PZA	13	8	3	3	1	2	2	0	2	0	0	10	3	5	1	2
(per cent resistant)			*	*	*	*		*	*	*	*		*	*	*	*
All regimens	125	39	13	15	7	6	43	20	16	10	7	82	33	31	17	13
(per cent resistant)			(33)	(39)	(18)	(15)		(47)	(37)	(23)	(16)		(40)	(38)	(21)	(16)

* Percentage not calculated when number of patients in group is less than 12.

† *See* footnote to table 1.

tion of drug. The incidence of complete susceptibility may be derived by subtracting the sum of the two grades of drug resistance from the total number of patients with data available. The highest rates for the emergence of viomycin resistance were in the patients treated with viomycin alone and with viomycin combined with oxytetracycline or pyrazinamide. The rates were lowest in the grouped regimens in which viomycin was used with either streptomycin or isoniazid and other drugs. The rate for the group of regimens in which viomycin was used with PAS and other drugs was approximately that for the entire group of 125 patients (with data on 82 patients). This over-all rate for *in vitro* resistance to 10γ of viomycin per ml. was 33 per cent (complete resistance) with four months or less of therapy, and 47 per cent with more than four months of therapy. The rate for complete resistance to 100 γ per ml. was approximately one-half that for 10 γ per ml. in each instance.

TABLE 15

SURVIVAL OF PATIENTS TREATED WITH VIOMYCIN

(Life-Table Method)

(All Regimens)

Period (in Months After Start of VM)	Number of Patients Entering Period	Lost Alive (One Half Period of Exposure)	Person Periods of Exposure	Number of Patients Died in Period	Per cent Died in Period	Per Cent Lived Through Period	Expectation of Survival per 100 Patients
1–4	125	7.5	117.5	7	5.96	94.04	94
5–8	103	17.5	85.5	1	1.17	98.83	93
9–12	67	10.0	57.0	3	5.26	94.74	88
13–18	44	6.5	37.5	4	10.67	89.33	79
19–24	27	2.5	24.5	2	8.16	91.84	73

TABLE 16

SURVIVAL OF PATIENTS TREATED ON VARIOUS VIOMYCIN REGIMENS

(Calculated by Life-Table Method)

Expectation of Survival per 100 Patients, Months After Start of Viomycin Therapy

Regimen*	4	8	12	18	24
VM alone	93	93	88	72	66
VM-PAS	96	96	96	96	96
VM-INH	93	93	82	82	—
Grouped regimens					
VM-PAS-others	98	98	98	98	98
VM-SM-others	94	94	75	75	75
VM-INH-others	95	95	87	87	—
VM-OTC and VM-PZA	91	80	80	62	41
All regimens	94	93	88	79	73

* *See* footnote to table 1.

ACHIEVEMENT OF INACTIVE AND ARRESTED STATUS DURING AND AFTER VIOMYCIN THERAPY
By 1950 NTA Diagnostic Standards

Regimen†	Duration of Observation																	
	1–4 Months			5–8 months			9–12 Months			13–18 months			19–24 Months			All Durations		
	Number of Patients Observed	Inactive	Inactive and Arrested	Number of Patients Observed	Inactive	Inactive and Arrested	Number of Patients Observed	Inactive	Inactive and Arrested	Number of Patients Observed	Inactive	Inactive and Arrested	Number of Patients Observed	Inactive	Inactive and Arrested	Number of Patients Observed	Inactive	Inactive and Arrested
VM alone	31	0	2	27	1	5	20	2	3	16	2	2	13	2	2	31	2	6
(per cent)		(0)	(6)		(4)	(19)		(10)	(15)		(12)	(12)		(16)	(16)		(6)	(19)
VM-PAS	27	0	3	23	2	3	18	1	2	17	1	3	10	1	1	27	2	5
(per cent)		(0)	(11)		(9)	(13)		(6)	(11)		(6)	(18)		*	*		(7)	(19)
VM-INH	30	0	6	22	3	9	12	2	5	4	1	1	—	—	—	30	5	12
(per cent)		(0)	(20)		(14)	(41)		(17)	(42)		*	*	—	—	—		(17)	(40)
VM-SM	5	0	0	4	0	2	2	0	1	—	—	—	—	—	—	3	0	3
VM-INH-SM	3	0	0	3	0	0	2	0	0	—	—	—	—	—	—	3	0	0
VM-INH-PAS	6	0	4	6	4	4	3	3	3	—	—	—	—	—	—	6	4	4
VM-SM-PAS	2	0	0	2	0	1	2	1	1	2	1	1	—	—	—	2	1	1
VM-INH-SM-PAS	8	0	1	7	1	4	1	0	1	—	—	—	—	—	—	8	1	5
VM-OTC	11	0	0	8	0	0	6	0	0	4	0	0	3	0	0	11	0	0
VM-PZA	2	0	0	1	0	0	1	0	0	1	0	0	—	—	—	2	0	0
Grouped regimens																		
VM-PAS-others	43	0	8	38	7	12	24	5	7	19	2	4	10	1	1	43	8	15
(per cent)		(0)	(19)		(18)	(32)		(21)	(29)		(11)	(21)		*	*		(19)	(35)
VM-SM-others	18	0	1	16	1	7	7	1	3	2	1	1	—	—	—	18	2	9
(per cent)		(0)	(6)		(6)	(44)		*	*		*	*		—	—		(11)	(50)
VM-INH-others	47	0	11	38	8	17	18	5	9	4	1	1	—	—	—	47	10	21
(per cent)		(0)	(23)		(21)	(45)		(28)	(50)		*	*		—	—		(21)	(45)
VM-OTC and VM-PZA	13	0	0	9	0	0	7	0	0	5	0	0	3	0	0	13	0	0
(per cent)		(0)	(0)		*	*		*	*		*	*		*	*		(0)	(0)
All regimens	125	0	16	103	11	28	67	9	16	44	5	7	26	3	3	125	15	36
(per cent inactive and arrested)		(0)	(13)		(11)	(27)		(13)	(24)		(11)	(16)		(12)	(12)		(12)	(29)

* Percentages not calculated when number of cases in group is less than 12.
† *See* footnote to table 1.

TABLE 18

SELECTED RESULTS OF VIOMYCIN THERAPY

(By Grouped Regimens, in Per Cent)

(Eight Months After Start of Therapy)

Regimens*	Moderate and Marked Roentgenographic Improvement	Cavity Closed (by Roentgenographic Observation)	Relapse (by Roentgenogram)	Bacteriologic Conversion (by Culture)	Complete Susceptibility to 10 γ of VM per ml.	Survival†	Achievement of Inactive or Arrested Status
VM alone	7	18	37	19	0	93	19
VM-PAS-others	18	27	11	37	(30)	98	32
VM-SM-others	31	(36)	13	50	(40)	94	44
VM-INH-others	16	33	18	48	29	95	45
VM-OTC and VM-PZA	(11)	(0)	(44)	(11)	(0)	(80)	(0)
All regimens	14	24	25	31	16	93	27

* *See* footnote to table 1.

† Percentages calculated by life-table method. ( ) Percentages in parentheses based on less than 12 cases.

General Outcome

Survival: Survival rates have been calculated by the life-table method for all viomycin-treated patients, as indicated in table 15.[6] In table 16 may be seen the figures for expectation of survival at each observation point per 100 patients for each regimen and group of regimens, which indicate that the lowest rate of survival was among those treated with viomycin alone or with viomycin with either oxytetracycline or pyrazinamide. Rates for the grouped regimens of treatment with streptomycin–isoniazid and other drugs are intermediate, and the highest survival rate (98 per cent) was in the group treated with viomycin–PAS and other drugs.

Achievement of inactive or arrested status: Investigators were asked to report on the achievement of inactive or arrested status of the tuberculous lesions, as defined in the 1950 edition of the *Diagnostic Standards* of the National Tuberculosis Association (NTA), and the results are given in table 17. Only 15 patients (12 per cent) achieved an inactive status of their lesions; in an additional 21 (17 per cent), the tuberculosis became arrested, making a total of 36 (29 per cent) with either inactive or arrested disease. The rates in general were considerably higher for groups of patients treated with multiple-drug therapy than in those treated with viomycin alone, except that there was no instance of achieving either

[6] Three nontuberculous deaths are excluded: (*1*) a patient treated with viomycin-isoniazid for seven months who died at that point from a massive gastrointestinal hemorrhage associated with a peptic ulcer; (*2*) a patient treated with viomycin-streptomycin for four months who died two days after a segmental pulmonary resection twelve months after the start of viomycin therapy; and (*3*) a patient treated with viomycin-streptomycin-PAS for thirteen months who died at that point following a pneumonectomy. Including these deaths, the crude death rate is 16 per cent (20 deaths).

an arrested or inactive status of the tuberculosis in the group of 13 patients who received viomycin with either oxytetracycline or pyrazinamide.

Summation of observations: For convenience and perspective, in table 18 are brought together selected observations on results for certain treatment groups at the end of eight months of observation (from tables 8 through 17). Uniformly the best results were obtained in three "grouped regimens" (viomycin–PAS and others, viomycin–streptomycin and others, viomycin–isoniazid and others), and the results were the poorest in the group treated with viomycin alone or with viomycin and either oxytetracycline or pyrazinamide. A similar tabulation made at the end of twelve months of observation, with data on fewer cases available, would show almost exactly the same relative positions. Among the three "grouped regimens" with the best results, there was no consistent pattern, according to the different observations, as to which was superior.

DISCUSSION

The nature of the present study does not make it possible to determine with precision which combination of drugs given with viomycin, in the type of patient treated, leads to the best results. The choice of antimicrobial regimen was not made by random selection; in many instances a small number of patients were treated with one combination of drugs. Moreover, analysis of such background factors as previous antimicrobial therapy, streptomycin resistance, stage and trend of disease, and other factors (tables 2 and 3) shows that the treatment groups were not strictly comparable, although they were relatively so. In general, the patients treated with viomycin alone and with viomycin and either oxytetracycline or pyrazinamide had somewhat less favorable disease at the start of therapy as determined by such defining characteristics. Nevertheless, the evidence strongly suggests that, in roughly comparable cases, better results were obtained when the viomycin was accompanied by PAS, streptomycin, or isoniazid, or by various combinations of these other drugs. In general, such additional drugs were administered in relation to the known absence of prior administration of the same drugs and of known or presumed drug resistance to them *in vitro*. Although the data are limited, there is a strong suggestion that other potent antimicrobials when available should be used with viomycin when there is an indication to administer this antimicrobial. The results of combined therapy using oxytetracycline are disappointing, and it is possible that this relatively weak antituberculous drug, although apparently effective in delaying the emergence of streptomycin resistance when given with streptomycin (25, 26), may not be as effective when administered in combination with viomycin.[7]

By its nature, this study of the effects of viomycin therapy in the re-treatment

[7] One of the participating hospitals (Madison) reports that it has treated a small number of patients, with far advanced disease, bacteriologically infectious, and with laboratory and clinical evidence of resistance to streptomycin, isoniazid, and PAS, with 2 gm. of oxytetracycline a day and the usual dose of viomycin for three months; on this regimen there has been unfavorable progression of the disease in no patient, slight roentgenographic improvement in one, and a reversal of infectiousness by smear in 4.

of pulmonary tuberculosis after failure of previous antimicrobial therapy does not permit other than relatively crude retrospective comparisons with the results of re-treatment with other antimicrobials. It has been quite clearly shown (27) that the results of re-treatment of streptomycin-resistant tuberculous infections are considerably less satisfactory than the results of re-treatment of streptomycin-susceptible infections. This is particularly true when the comparison is made with tuberculosis previously untreated with any chemotherapy. When isoniazid alone was used in 1952 in the treatment of streptomycin-resistant infections (with tubercle bacilli resistant to 10 γ per ml. *in vitro*) (22), the results after four months of treatment with respect to roentgenographic improvement and reversal of infectiousness were comparable to those reported here with viomycin, as may be seen from the following:

	Per cent of all degrees of roentgenographic improvement	*Per cent of moderate and marked roentgenographic improvement*	*Per cent of "conversion" of sputum and/or gastric lavage specimen*
Isoniazid (1952)	40	8	19
Viomycin and others (1954)	39	6	14
Viomycin alone (1954)	20	3	10

In the 1952 study of the re-treatment of streptomycin-resistant infections, moreover, the results were somewhat better with multiple-drug therapy than with isoniazid alone, as is the case in the present series with viomycin. In agreement with other observers (14, 15), it is probable from these data that the antituberculous effect of viomycin is less than that of streptomycin or isoniazid.

Whereas in the present series the incidence of any one manifestation of toxicity to viomycin was not frequent (table 7), the apparent necessity, in the opinion of those responsible for the treatment, to discontinue viomycin therapy in 14 per cent of the cases is not to be taken lightly. Allergic manifestations (fever, angioneurotic edema, and rash), renal irritation and toxicity (impairment of renal function, severe albuminuria, and electrolytic disturbances), nausea and vomiting, and toxicity for the two branches of the eighth cranial nerve were all of approximately equal incidence. Of more than passing interest is the observation that, in spite of the fact that 1 gm. of streptomycin and 2 gm. of viomycin were injected on the same day, twice a week, in 18 patients treated for an average duration of 6.4 months, only one patient showed significant impairment of vestibular function, and none showed impairment of auditory function.

The data on toxicity to viomycin in this series substantiates the observations made by others that, when viomycin is given on an intermittent schedule, twice a week, it can be tolerated by most patients, in contrast to its daily administration, but that toxicity remains a problem and careful laboratory observation is indicated throughout the entire period of viomycin therapy.

Data have been included on the emergence of tubercle bacilli resistant to viomycin at the conclusion of viomycin therapy in 82 patients (table 14): after four months or less of viomycin therapy in 39 patients and after more than four months of therapy in 43 patients. Addition of other effective antimicrobial drugs to viomycin appeared clearly to be related to a lower rate of emergence of viomy-

cin-resistant tubercle bacilli. Nevertheless, in all instances the rates of resistant bacilli were higher with longer duration of treatment, and it is probable that the pattern of viomycin resistance is similar to that of streptomycin resistance. Among the 27 patients who experienced roentgenographic relapse during viomycin therapy, the status of viomycin susceptibility was known in 21. There was loss of viomycin susceptibility to 10 γ per ml. *in vitro* in 19 of these 21 patient-strains (90 per cent), with retention of susceptibility in 2 (10 per cent). In contrast, a loss of viomycin susceptibility occurred in the strains of 49 of the remaining 61 patients who had no roentgenographic relapse (74 per cent), and retention of drug susceptibility was present in 16 (26 per cent). The figures for the retention of susceptibility to 100 γ per ml. are 67 per cent for those with roentgenographic worsening and 62 per cent for those without roentgenographic relapse. Thus, there would appear to be some association between roentgenographic relapse and the loss of susceptibility *in vitro* to 10 γ of viomycin per ml. but not to 100 γ per ml.

It is interesting to note that, in the series of 23 patients treated by Schaffeld, Garthwaite, and Amberson (14), there were 4 roentgenographic relapses in 22 patients with pulmonary tuberculosis (18 per cent), and 3 among 14 patients who had had prior antimicrobial therapy (21 per cent). The incidence of roentgenographic relapse during viomycin therapy in the present series is 22 per cent (27 of 125 patients).

The severity of the therapeutic problem in the group of patients here reported cannot be overemphasized. Virtually all (98 per cent) had received previous antimicrobial therapy which had averaged a total of 17.4 months in duration. The patient-strains of tubercle bacilli were completely resistant to 10 γ of streptomycin per ml. in 76 per cent of the group. The pulmonary tuberculosis was far advanced in extent in 95 per cent of the group and, in 92 per cent, tubercle bacilli could be seen by microscopic examination of the sputum. The disease had been actively progressive immediately prior to viomycin therapy in 56 per cent. Multiple cavities were present in 60 per cent, and cavities exceeding 4 cm. in diameter were present in 57 per cent of the 125 cases. Hence, even with the best of antimicrobial therapy, relatively little might have been expected in the way of therapeutic results in such a group of tuberculous patients.

Viewed in this perspective, the facts that there should be roentgenographic improvement in approximately 50 per cent, cavity closure in approximately 25 per cent, sputum "conversion" (culture) in approximately 30 per cent, achievement of inactive or arrested status of the disease in approximately 30 per cent and a gross mortality of not more than approximately 15 per cent represent a not inconsiderable achievement under the circumstances. It is not readily apparent from this analysis exactly how much viomycin of itself has contributed to the results, for it will be noted that in 66 patients, after viomycin therapy was terminated, additional antimicrobial therapy was considered to be necessary and was administered in 54 (88 per cent); and certainly "the power of the body to heal a lesion depends as well on other factors" as upon antimycobacterial agents (14). But it may conservatively be stated from this evidence that viomycin was

at least helpful, along with other factors, in controlling extensive pulmonary tuberculous disease after the "failure" of other therapy. It seems apparent that viomycin, probably in combination with other antimicrobials, will have a place in the treatment of such therapeutic problems, at least until other more effective agents become available.

It will be noted that this report consists of results from the treatment of 125 patients with viomycin in sixteen different hospitals, with an average of less than 8 patients per hospital. Uniformity of management, observation, and reporting has made possible an analysis which few hospitals might by themselves have been able to carry out. This type of analysis has obvious limitations, however, such as the inevitable lack of complete uniformity of interpretation of clinical observations by the many staff members participating, and even of laboratory determinations. The strength of this type of analysis lies in the number of cases reported. Such a report must always be considered to be supplementary to the more customary meticulous observation of individual patients by cohesive staffs of individual hospitals. Each such kind of investigation will have its merits and its defects, but each may be considered to complement the other to an appreciable degree. The present report on a fairly large series of treated patients has apparently served to confirm reports on the results of viomycin therapy which were cited earlier.

SUMMARY

A series of 125 patients with active pulmonary tuberculosis treated with viomycin in sixteen Veterans Administration hospitals is reported. They overwhelmingly represented cases of advanced, highly cavernous, "sputum-positive", progressive disease, the failures of much prior antimicrobial therapy.

Viomycin was administered in a dose of 2 gm. a day twice a week for an average of 5.7 months. Thirty-one patients were treated with viomycin alone; 27, with viomycin–PAS (12 gm. daily); 30, with viomycin–isoniazid (300 mg. daily); and the remaining 37, on seven other antimicrobial regimens. By grouping patients treated on regimens with a common denominator (viomycin with PAS, streptomycin, or isoniazid), groups of sufficient size for analysis were obtained which are reasonably comparable with respect to defining characteristics.

The incidence of toxic manifestations was determined by a series of careful laboratory procedures carried out at frequent intervals. A total of 53 manifestations was observed in 41 patients (33 per cent of the series), and in 18 patients (14 per cent) it was judged to be necessary to discontinue the viomycin because of such toxicity. The incidence of serious toxic manifestations was approximately equally divided between renal, allergic, impairment of function of the eighth cranial nerve, and nausea and vomiting, with no one toxic manifestation necessitating discontinuation of therapy in more than 3 instances (2.4 per cent).

Observations of clinical results were obtained in approximately the following percentages for the series as a whole: roentgenographic improvement, 50 per cent; cavity closure, 25 per cent; sputum "conversion" by culture, 30 per cent; survival, 85 per cent; and achievement of arrested or inactive status of the tuberculosis, 30 per cent.

The results were almost uniformly superior when viomycin was administered with one or more of the commonly used other antimicrobial agents (isoniazid, streptomycin, or PAS). The poorest results were obtained in the groups treated with viomycin alone and viomycin with either oxytetracycline or pyrazinamide.

Roentgenographic relapse occurred during viomycin therapy in 22 per cent of the patients, and in 31 per cent during and after viomycin therapy, during a total of 122.5 patient-years of observation (0.32 relapse per patient per year). There appeared to be an association between loss of susceptibility of the patient's tubercle bacilli to 10 γ of viomycin per ml. *in vitro* and in the incidence of such relapse.

The results of viomycin therapy in this series of re-treatment "streptomycin-resistant" patients are roughly comparable to other series of such re-treatment cases.

It is concluded that, when there is a clinical indication for antimicrobial therapy in tuberculosis and the more commonly employed agents (isoniazid, streptomycin, PAS) are not available for any reason, viomycin may be employed as an effective antimicrobial in the treatment of tuberculosis. Viomycin is less effective than streptomycin or isoniazid. Moreover, the toxicity of viomycin, even on a schedule of administration of 2 gm. a day twice a week, necessitates careful laboratory control.

SUMARIO

Re-Tratamiento de la Tuberculosis Pulmonar Avanzada con Viomicina

La serie presentada comprende 125 enfermos con tuberculosis pulmonary activa tratados con viomicina en dieciséis hospitales de la Administración de Veteranos. Representan en su inmensa mayor a casos de enfermedad evolutiva, muy avanzada, sumamente cavernosos y con esputo positivo, fracasos de muche terapéutica antimicrobiana anterior.

La viomicina fué administrada en una dosis de 2 gm. al día dos veces semanales, durante un promedio de 5.7 meses. Treinta y un enfermos fueron tratados con viomicina sola; 27 con viomicina y PAS (12 gm. diarios); 30 con viomicina e isoniacida (300 mg. diarios); y los 37 restantes con otros siete regímenes antimicrobianos. Agrupando a los sujetos tratados con regímenes que tenían un denominador común (viomicina-PAS, estreptomicina o isoniacida), obtuviéronse grupos de suficiente magnitud para análisis, que son bastante comparables con respecto a características determinantes.

Se determinó la incidencia de manifestaciones tóxicas con una serie de procedimientos de laboratorio ejecutados a plazos frecuentes. Se observó un total de 53 manifestaciones en 41 enfermos (33 por ciento de la serie), y en 18 enfermos (14 por ciento) se juzgó necesario discontinuar la viomicina por virtud de dicha toxicidad. La incidencia de manifestaciones tóxicas graves se dividió aproximadamente por igual entre renales, alérgicas, disfunción del octavo par craneal y náuseas y vómitos—con sólo una manifestación tóxica que impuso el abandono de la medicación en 3 casos (2,4 por ciento).

Las observaciones de los resultados clínicos dieron aproximadamente los siguientes valores para la serie en conjunto: mejoría radiográfica, 50 por ciento; cierre de cavernas, 25 por ciento; "viraje" del esputo en cultivos, 30 por ciento; sobrevivencia, 85 por ciento; y obtención de estacionamiento o inactividad, 30 per ciento.

Los resultados fueron casi siempre superiores cuando se administraba la viomicina con uno o más de los otros agentes antimicrobianos usados corrientemente (isoniacida, estreptomicina o PAS), y peores en los grupos tratados con viomicina sola o viomicina con oxitetraciclina o piracinamida.

Descubriéronse recidivas con la observación radiográfica durante la viomicinoterapia en 22 por ciento de los enfermos, y en 31 por ciento durante la viomicinoterapia y después, durante un total de 122.5 pacientes-años de observación (0.32 recidivas por enfermo al año). Al parecer había una asociación entre la pérdida de susceptibilidad a 10-γ de miocina por ml. in vitro y la incidencia de dichas recidivas.

Los resultados de la viomicinoterapia en esta serie de enfermos "estreptomicinorresistentes" se comparan toscamente con los obtenidos en otras series de casos semejantes de re-tratamiento.

Dedúcese que, cuando existe una indicación clínica para terapéutica antimicrobiana antituberculosa y por alguna razon no hay disponibles los agentes empleados más comúnmente (isoniacida, estreptomicina, PAS), cabe emplear la viomicina como antimicrobiano eficaz en el tratamiento de la tuberculosis, aun siendo menos eficaz que la estreptomicina y la isoniacida. Sin embargo, su toxicidad, aun con un régimen posológico de 2 gm. diarios dos veces semanales, impone cuidadosa comprobación de laboratorio.

RESUME

Re-traitement par la viomycine de cas avancés de tuberculose

Rapport sur une série de 125 cas de tuberculose pulmonaire en activité traités par la viomycine dans seize hôpitaux de la "Veterans Administration." Ces cas illustrent complètement la maladie évolutive au stade très avancé avec lésions cavitaires marquées et crachats positifs, ils représentent les échecs de la thérapie antibactérienne antérieure.

La viomycine était administrée à la dose de 2 grammes par jour, deux fois par semaine, pendant une période moyenne de 5, 7 mois. Trente et un malades furent traités exclusivement par la viomycine; 27 par la viomycine et le PAS (12g par jour); 30 par la viomycine et l'isoniazide (300 mg par jour); les 37 autres, par sept autres médications antibactériennes. Le groupement des sujets traités par les médications ayant un dénominateur commun (viomycine-PAS, streptomycine, ou isoniazide) a permis d'obtenis des groupes d'importance suffisante pour permettre l'analyse, et suffisamment comparables à l'égard de la définition des caractéristiques.

L'incidence des manifestations toxiques fut déterminée par une série de procédés de laboratoire soigneusement exécutés et fréquemment répétés. Un total de 53 réactions a été enregistré chez 41 patients (33% de la série) et chez 18 d'entre eux (14%) l'arrêt de la viomycine a été jugé nécessaire en raison de l'intolérance. L'incidence des effets secondaires sévères se répartissait à peu près également entre: les réactions rénales, l'allergie, l'altération de la fonction de la huitième paire cranienne, les nausées et vomissements—à plus de 3 reprises (2, 4%) l'intolérance a nécessité l'interruption du traitement.

Les observations relatives aux résultats cliniques, pour l'ensemble de ces séries, ont permis d'obtenir les pourcentages approximatifs suivants: amélioration radiologique, 50%; oblitération cavitaire, 25%; "conversion" des crachats en culture, 30%; survie 85%: stabilisation ou inactivation 30%.

Les résultats se sont montrés presque constamment supérieurs lorsque la viomycine était administrée avec l'un ou plusieurs des autres agents antibactériens communément utilisés (isoniazide, streptomycine, ou PAS). Les résultats les moins favorables ont été obtenus dans les groupes traités exclusivement par la viomycine, ou par la viomycine asociée à l'oxytétracycline ou à la pyrazinamide).

La rechute, vérifiée par l'observation radiologique, a été observée au cours de la viomycine-thérapie chez 22% des malades et, dans 31% des cas, au cours ou après la viomycine-thérapie, pendant une période d'observation totale de 122,5 malade-année (0,32 rechutes par malade par an). Il paraît exister une relation entre la perte de la sensibilité à 10 de viomycine par ml. *in vitro* et l'incidence de ces rechutes.

Les résultats de la viomycine-thérapie dans cette série de re-traitement des malades "streptomycine-résistants" sont grossièrement comparables à ceux des autres séries de re-traitement de cas analogues.

En conclusion, lorsqu' il existe une indication clinique de thérapie antibactérienne de la tuberculose et que, pour une raison quelconque, on ne peut disposer des agents les plus habituellement utilisés (isoniazide, streptomycine, PAS) la viomycine, quoique moins efficace que la streptomycine ou l'isoniazide, peut être employée comme antibactérien efficace dans le traitement de la tuberculose. Toutefois, en raison de sa toxicité, même à la dose de 2 grammes par jour, deux fois par semaine, son administration réclame un contrôle méticuleus au laboratoire.

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ADRENAL CORTICAL FUNCTION DURING ISONIAZID THERAPY FOR PULMONARY TUBERCULOSIS[1,2,3]

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INTRODUCTION

Participation of adrenal mechanisms must now be included in any consideration of the pathologic physiology of infection. Those determinants of the total body reaction to disease presently known to be mediated via adrenal cortical influences embrace such otherwise diversified phenomena as antibody formation; pyretic response; growth, activation, and dissolution of lymphoid tissue; as well as nonspecific resistance itself. Of historic and fundamental concern in this problem have been the early efforts to determine relationships between the adrenal gland and tuberculosis. In 1855, Thomas Addison (1) first described adrenal insufficiency due to tuberculosis. Although accumulated evidence (2) lays less emphasis on the tuberculous process as the major cause of Addison's disease, this observation served to focus attention on the possible adrenal modification of the course of tuberculosis. The complex ramifications of this relationship were not fully appreciated until some eighty-five years later, when further investigation suggested that the adrenal was not necessarily intimately involved in the production of the electrolyte and other disturbances seen in pulmonary tuberculosis (3).

The characteristics of the favorable response of tuberculous patients during isoniazid therapy has increased the interest of the present writers in the possible metabolic and endocrine effects of this and chemically related compounds. Clinical observations of defervescence, increased appetite, weight gain, and euphoria out of proportion to objective improvement are strongly reminiscent of changes associated with corticotropin and cortisone administration and suggest possible adrenal cortical effects, either as a primary action of isoniazid or as secondary consequences. The present study deals with observations of adrenal cortical function in patients undergoing isoniazid therapy for pulmonary tuberculosis. The prevailing concept of chemotherapy utilizing multiple-drug regimens is no longer conducive to extensive observations on patients who receive isoniazid alone. For this reason, the present project is not likely to be reproduced and should offer important information with respect to the extrachemotherapeutic actions of isoniazid and, specifically, to the role of the adrenal cortex in the mediations of such effects.

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METHODS

The patients observed were selected at random from alternate admissions to the Tuberculosis Service of the Albany Hospital. Eight patients participated in the study. The status of the pulmonary disease in all 8 patients was approximately the same, as noted in table 1. The disease in patient M. A. reverted to an arrested status four months after therapy. There were no signs or symptoms referable to involvement of extrapulmonary structures, nor were there any intercurrent infections, diseases, or complications noted in any of the patients during the study. A plain film of the abdomen was obtained to rule out calcification of the adrenal glands. All patients were hospitalized throughout the study and followed the customary program of rest and a nutritious diet. No specific antituberculous therapy other than isoniazid was given. The administration of 3 to 5 mg. of isoniazid per kg. of body weight represented the initial form of chemotherapy in these patients. The period of observation ranged from four to twenty weeks.

TABLE 1

A Comparison of Fasting Eosinophils and the Urinary Uric Acid Creatinine (UA/Cr) Ratio Before and After Isoniazid

Patient	Age and Sex	Classification of Tuberculosis*	Days of Isoniazid Therapy	Before Isoniazid		After Isoniazid	
				Eosinophils Number per Cu.Mm.	UA/Cr Ratio	Eosinophils Number per Cu.Mm.	UA/Cr Ratio
M. A.	49 F	Active II	140	75	0.70	102	0.43
H. C.	59 M	Active I	26	35	0.88	110	0.73
M. F.	47 M	Arrested IV	14	219	0.51	331	0.34
T. F.	47 M	Arrested III	86	156	0.49	200	0.41
A. M.	37 F	Arrested III	120	143	0.50	156	0.38
J. M.	33 M	Arrested III	14	44	0.43	207	0.46
J. O.	16 F	Arrested III	14	200	0.52	337	0.41
J. P.	63 M	Arrested II	14	82	0.68	150	0.59

* Diagnostic Standards of the National Tuberculosis Association (1950 edition).

All patients were weighed weekly, and careful notation was made of clinical as well as subjective changes. Evaluation of adrenocortical function was ascertained by the periodic observation of the metabolic consequences of an intravenous administration of 20 international units of corticotropin[4] in 500 ml. of 5 per cent glucose in distilled water during an eight-hour period (4). Measurements were made of the urinary excretion of 17-ketosteroids, 17-hydroxycorticoids, uric acid, and creatinine on the day prior to, during, and after the administration of corticotropin. Total circulating eosinophil counts were obtained in the fasting state and four, eight, and twenty-four hours after starting the corticotropin infusion. Similar determinations with and without corticotropin were made at spaced intervals. Food was withheld for a period of at least two hours prior to obtaining blood specimens. 17-Hydroxycorticoids were measured according to the method outlined by Reddy, Jenkins, and Thorn (5), and 17-ketosteroids were determined in the conventional manner (6). The above procedures, as well as liver and kidney function tests, were performed routinely prior to and during therapy.

OBSERVATIONS

Most of the patients exhibited the characteristic subjective responses associated with isoniazid therapy, viz., increased appetite, weight gain, improved

[4] Kindly supplied through the generosity of Dr. Albert Holland, Medical Director, Armour Laboratories, Chicago.

sense of well-being, reduction in fever, as well as objective evidence of improvement in the pulmonary lesions. Liver function (bromsulfalein retention, thymol turbidity) and kidney function tests showed no significant alterations.

Adrenal Cortical Function in the Resting State Before and During Therapy

Eosinophils: Prior to isoniazid therapy, it will be noted that the fasting total circulating eosinophil level ranged from 35 to 219 per cu. mm. (table 1). As therapy progressed, there was a tendency toward a progressive rise in the fasting eosinophil count of all patients. This was most pronounced in patient J. M., whose total eosinophil count increased from 44 to 207 per cu. mm. Of more significance, the initial response to corticotropin, as reflected by the eight-hour percentage fall, ranged from 48 to 100 and averaged 81 per cent, indicating adequate adrenal cortical responsiveness before receiving any treatment (table 2).

Urinary uric acid–creatinine ratios: Similarly, the urinary uric acid-creatinine

TABLE 2

MEASUREMENTS OF BASAL ADRENAL CORTICAL FUNCTION AND THE EFFECT OF CORTICOTROPIN STIMULATION DURING ISONIAZID THERAPY

Patient	Days of Isoniazid Therapy	Total Isoniazid	Weight	Eosinophils				17-Ketosteroids	
				Control	Per Cent Change After Corticotropin			Control	Per Cent Change After Corticotropin
					4 Hours	8 Hours	24 Hours		
		gm.	*pounds*	*no./mm³*				*mg. per 24 hours*	
C. F.	−4	0.0	152	156	+19	−48	+28	18.9	+18.6
	+10	3.5	154	225	−94	−100	+39	17.0	+51.0
	+51	17.9	160	228	−92	−100	+8	19.8	+56.0
	+58	20.3	161	246	−95	−100	+5	17.2	+31.0
	+65	22.7	160	208	−90	−100	+16	16.6	+15.4
	+72	25.2	162	246	−93	−100	−16	16.6	+38.0
	+86	27.1	162	200	−84	−96	+18	17.2	+38.0
M. A.	−3	0.0	123	75	−27	−96	—	8.1	+32.0
	+9	2.5	121	66	−77	−76	+21	10.5	+46.0
	+18	5.0	121	111	−89	−100	−38	10.7	+57.0
	+46	12.7	124	87	−63	−93	+45	8.4	+62.0
	+85	23.4	128	108	−78	−93	+18	9.8	+14.0
	+92	25.3	129	72	−86	−100	−14	10.8	+26.0
	+99	27.2	129	96	−81	−94	+14	12.8	+7.0
	+141	38.8	131	102	−72	−90	—	10.2	+25.0
A. M.	−1	0.0	100	143	−71	−100	+17	6.6	+113.0
	+64	14.4	107	133	−78	−100	−43	15.4	+6.5
	+71	15.9	108	146	−75	−95	+9	12.4	+38.0
	+78	17.5	109	152	−79	−100	−8	6.4	+53.0
	+85	19.1	108	161	−87	−100	−12	7.9	+40.0
	+99	22.7	109	165	−80	−96	+7	9.5	+31.0
	+120	27.0	108	156	−86	−100	−10	5.9	+147.0

ratios (UA/Cr) varied from 0.43 to 0.88 before institution of therapy. There was a consistent rise in this value following corticotropin administration, substantiating the presence of adequate adrenal cortical activity before therapy. Of considerable interest was the observation that this ratio tended to fall as therapy was continued, the amount of reduction apparently being related to the degree of clinical improvement observed as well as to the duration of treatment. Thus, patient M. A., who had the best objective evidence of improvement, showed a 39 per cent reduction in the urinary uric acid-creatinine ratio at the end of twenty weeks of isoniazid treatment. The condition of patient A. M. improved less markedly, a 24 per cent fall in the urinary uric acid-creatinine ratio being noted at the end of seventeen weeks. This correlation also obtained during the first two weeks of therapy as in the case of J. O., who had a 17 per cent fall in the UA/Cr ratio and showed early improvement, as opposed to J. M., who had a rise of 11 per cent and whose disease process was not ameliorated by isoniazid during the period studied. The significance and importance of these observations with respect to their possible use as a prognostic sign, or indication of improvement, will be discussed below.

17-Ketosteroid excretion: The pretherapy urinary 17-ketosteroid excretion was also within normal limits, ranging from 6.6 mg. to 18.9 mg. per twenty-four hours. The response to corticotropin was inconstant and varied from plus 18.6 per cent to plus 113 per cent. Throughout the course of therapy, individual variations were noted in 17-ketosteroid excretion, but no consistent patterns were observed. The most striking change was noted in patient A. M., who more than doubled her 17-ketosteroid excretion after two months of isoniazid therapy. This returned to pretreatment levels in the succeeding weeks. The urinary 17-hydroxycorticoids ranged from 3.3 to 7.1 mg. per twenty-four hours before therapy and varied slightly above and below control values, but showed no consistent pattern during isoniazid administration.

Adrenal Cortical Function During Isoniazid Therapy as Determined by Corticotropin

The potential of adrenal cortical responsiveness was measured by repeated eight-hour intravenous corticotropin tests at varying intervals throughout the period of isoniazid administration (table 2). The percentage changes in circulating eosinophils and urinary 17-ketosteroid excretion were used as the primary indices. The least variability was observed in the eight-hour eosinophil response, which showed a consistent fall of 90 to 100 per cent. The four-hour response showed a fairly consistent depression, averaging greater than 50 per cent, while the twenty-four-hour percentage change ranged from minus 43 to plus 45. The significance of the latter observation will be discussed later.

Unlike the persistent pattern observed in eosinophil levels following corticotropin administration, the percentage change in 17-ketosteroids showed a consistent but less predictable response. For example, the percentage change in 17-ketosteroids following corticotropin administration in patient A. M. varied from plus 6.5 to plus 147, whereas the eight-hour eosinophil reduction varied

from 95 to 100 per cent. When the initial 17-ketosteroid percentage response to corticotropin is compared individually with the average of subsequent 17-ketosteroid responses during therapy, it is observed that patient T. F. averaged a 38.2 per cent increase in 17-ketosteroid excretion; patient M. A., a 4 per cent change; and patient A. M., a 53 per cent decrease. In other words, in one patient the per cent increase was greater, in one it was unchanged, and in the third it was less than the initial response to corticotropin prior to instituting isoniazid. This indicates that such determinations bore little relationship to the course of the disease. In other respects, however, correlation between the various tests and clinical improvement was reasonably consistent and informative.

DISCUSSION

Of specific interest in the present study has been the objective evaluation of adrenal cortical function during isoniazid therapy. As indicated in the introduction, past observations suggested that adrenal factors might form the basis for certain subjective responses observed. With recent improvement in biochemical and biologic criteria, it becomes possible to obtain concrete information with which to substantiate or negate the theoretic implications of drug-induced adrenal modifications of the phthisic state. The need for such criteria is evident from a consideration of specific changes recorded during isoniazid therapy. The euphoric response (7), the ravenous appetite, the marked gain in weight, the reduction in temperature and erythrocyte sedimentation rate (7, 8, 9), reports of glycosuria (10), alteration in tuberculin sensitivity (11) and in drug responsiveness (9, 12), and relief of allergic rhinitis (9) are all reminiscent of increased adrenal cortical activity. On the other hand, the development of hypotension (12, 13), reduced tolerance to certain drugs (9, 12), eosinophilia (12, 14, 15), joint pains (15), pruritis (16, 17), drug fever (18), and other allergic manifestations (13, 17), together with loss of libido (16) and lack of significant effect on basal metabolic rate (19, 20) and the electroencephalogram (9) would suggest a different and possibly opposite state.

In the studies described above, the resting state of adrenal cortical function varied but slightly throughout the period of observation. One aspect worthy of attention was the tendency toward a progressive rise in fasting eosinophil levels during the period of isoniazid therapy. This is of some significance in view of reports that a rising eosinophil count represents a favorable prognostic sign in the course of treatment of, or recovery from, tuberculosis (21, 22). It is to be remembered that this trend should not necessarily be interpreted as a reflection of altered adrenal cortical activity. Except possibly for an eosinophil level of very low proportions, i.e., less than 50 per cu. mm., elevations and reductions of eosinophils when determined as isolated measurements may not be interpreted as the result of fluctuations in adrenal cortical activity. For, many other factors enter into the production and disappearance of eosinophils (2). Nevertheless, the level of circulating eosinophils is often considered to be related inversely to glucocorticoid production, and the lack of significant change in these corticoids in the patients observed militates against a primary effect of

isoniazid on basal adrenal cortical function as reflected in this aspect. Of more significance is the lack of change in urinary 17-ketosteroid excretion, a much more dependable and precise measure of adrenal cortical function. The 17-ketosteroid and 17-hydroxycorticoid excretion did not show any consistent pattern and adds further support to the opinion expressed here that isoniazid does not have any significant physiologic effect per se on adrenal cortical function.

Of much greater importance in interpreting the effects of isoniazid on adrenal cortical function are the observations obtained by serial corticotropin stimulations, which represent a more significant barometer of adrenal cortical potential. Thus, it is conceivable that the potential for response (the "stress response") might be altered by a given substance such as isoniazid, and yet not be apparent from measurements of resting function. The "stressor" in this instance is represented by corticotropin. It is to be remembered that, unlike the intramuscular corticotropin test, the intravenous corticotropin test used in these studies makes use of the eight-hour eosinophil response as opposed to the four-hour level. In the patients studied, the four-hour response varied considerably, although it was generally greater than 50 per cent. On the other hand, the eight-hour levels were characterized almost uniformly by a maximum response and averaged 94.4 per cent. On the basis of the above determinations, adrenal cortical function would appear to be well within normal limits.

The eight-hour intravenous corticotropin test as opposed to other procedures possesses distinct advantages as a measurement of adrenal cortical activity. Single injections of corticotropin when given intramuscularly have a peak of action at four hours which gradually disappears during the next two to six hours. Intravenous corticotropin, when given as a constant eight-hour infusion, has its maximum effect at eight hours, this activity being almost completely dissipated in twenty-four hours. An occasional normal subject will have a greater than 50 per cent reduction in eosinophils at the end of twenty-four hours, but the majority show a return to the control level or exhibit the so-called "rebound phenomenon" with eosinophils exceeding the control level. The latter phenomenon signifies that the adrenal effect has disappeared, while the maintenance of a significant depression (fall in eosinophils of 50 per cent or more) represents a continuing production of adrenal hormone.

None of the isoniazid-treated patients had a significant twenty-four-hour eosinophil reduction. On a single occasion, in 2 patients, percentage reductions of 38 and 43 persisted. In all other instances the eosinophils returned to control levels or above. It is again concluded from such observations that the capacity of the adrenal cortex to respond has not been enhanced or otherwise significantly influenced by isoniazid therapy.

The variability shown in 17-ketosteroid excretion throughout the period of isoniazid therapy does not indicate any significant change that might be interpreted as an isoniazid effect. The changes that have been noted are more plausibly explained on the basis of factors such as increased food intake and the general improvement in body nutrition which occurred in these patients. It

was thought that, if the urinary uric acid-creatinine ratio showed a reduction during therapy, it might serve as an index of therapeutic response. As will be noted in table 1, serial studies appear to indicate such a trend, although a stepwise reduction did not persist in all cases throughout the observation period. This may be due to the relatively short period of observation, as well as the influence of uncontrolled factors affecting this relationship, and it is suggested that this index may prove valuable in evaluating therapeutic response and degree of subsidence of active disease when applied to patients receiving any form of treatment, whether as a drug or as the classical rest form of therapy. Theoretically, a progressive reduction in uric acid-creatinine ratios in such instances may represent a tendency toward decreased tissue breakdown as improvement in the disease process occurs. It may be said that any alterations in this ratio during isoniazid therapy are not the direct result of altered adrenal cortical function.

The conclusion that isoniazid therapy does not influence adrenal cortical function has been drawn from the above studies. This has an important bearing on many aspects of tuberculosis treatment and response. It indicates that there must be other factors, such as direct central nervous system and cerebral effects of isoniazid, to explain the euphoria and sense of well-being. Recent animal experiments (23) and clinical reports of psychosis (24) developing during isoniazid therapy suggest the above may be true. Any enhanced favorable response in tuberculosis which may be observed when isoniazid is added to streptomycin therapy would not appear to be mediated through the adrenal cortex. Early investigations with exogenous corticotropin and cortisone led to the conclusion that these agents could be responsible for the reactivation and dissemination of the tuberculous process (25, 26), whereas later work (27, 28, 29) intimates that adrenal hormones, in some instances these and other pituitary and adrenal hormones, STH (somatotrophic hormone) and DCA (desoxycorticosterone acetate) respectively, may be beneficial in enhancing the host's natural facilities for combatting this disease. The importance of this observation is to be appreciated when considering the beneficial effects of corticotropin (30) and cortisone (31) used in combination with other therapy in certain forms of tuberculosis. Suggestions that isoniazid might have an endocrine action were entertained when it was observed that isoniazid increased the insulin requirement in tuberculous patients with diabetes mellitus (32, 10, 33). Although different (34) and even opposing (35, 36) observations have also been made, it suffices to say that any change in insulin requirement, whether it is an increase or decrease, is apparently not due to an alteration in adrenal cortical function per se, but may, perhaps, represent a response to changes in the activity of the disease process as well as variations in caloric intake. The failure to observe that isoniazid exerts an effective "stress" effect on the adrenal cortex correlates with the lack of anatomic evidence of any adrenal gland effects (37), the unaltered serum electrolytes (20), and absence of endocrine changes (18, 20) during isoniazid administration (38).

On the other hand, it may plausibly be argued that the present report does not exclude the possibility that isoniazid does exert endocrine or adrenal in

fluence. The dosage used, the duration of therapy, and the number of patients studied may all be cited as factors insufficient to warrant a definite conclusion. Its chemical structure and the therapeutic action of its congeners both suggest a possible endocrine action for isoniazid. Indeed, rat experiments utilizing the criteria of dextran-induced edema and formalin irritation (39) indicate that isoniazid in different concentrations has potent antiphlogistic properties akin to those of cortisone, and that this effect is actually on a nonspecific "stressor" basis and mediated via "hypophyseal-adrenal" pathways (39).

It must be said, however, that, while these observations have been confirmed in mice by Spain (40), the ability of isoniazid to exert a nonspecific delaying action on the early acute inflammatory response to oil-of-turpentine injections does not appear to be mediated through the adrenal glands, for a similar effect was observed in intact and adrenalectomized animals by the same investigator. The possibility also exists that the biochemical and biologic methods for estimating adrenal cortical function in the present study, although the most refined and widely accepted to date, are yet not sufficiently sensitive or encompassing to detect such alteration of adrenal activity. Certainly, the euphoric response, weight gain, prompt defervescence, and other corticomimetic actions of isoniazid are not explainable or comprehendible on the basis of bacteriostatic properties alone.

The original object of this work, namely, to ascertain whether the adrenal cortex plays any role in the development of weight gain, euphoria, and sense of well-being, remains only partly answered. While these effects are apparently not produced on the basis of altered adrenal cortical function, their cause remains unexplained. It would appear that a number of factors, such as the effect on the psyche, reduction in toxicity due to infection, control of the infectious process combining to cause a restoration of appetite, the development of strength, and an increased caloric intake with accompanying positive nitrogen balance, may all contribute to the improvement observed, and that no isolated system may account for the rather universal beneficial effects observed with isoniazid.

SUMMARY

The nature of the host's response during isoniazid therapy for pulmonary tuberculosis suggested a possible adrenal cortical influence. Observation of 8 patients with pulmonary tuberculosis and extensive study of 3 of these during isoniazid therapy in a dosage of 3 to 5 mg. per kg. for twelve to twenty weeks was undertaken to further ascertain this possibility.

All patients showed noticeable subjective improvement in mood, as well as other nonspecific effects along with varying degrees of objective improvement.

Utilizing eosinophil levels, 17-ketosteroid, uric acid, creatinine, and 17-hydroxycorticoid excretion patterns, the level of adrenal activity was determined before and during isoniazid therapy. Adrenal cortical responsiveness was assessed by the eight-hour intravenous corticotropin test both before and during therapy. In no instance in either the resting or stimulated state was a significant or consistent deviation from the normal observed.

A gradual rise in circulating eosinophils occurred during isoniazid therapy. The urinary uric acid-creatinine ratio varied directly with the disease process, and further use of this as an index of the degree of disease activity is suggested. The possible significance and limitations of the present study are discussed.

SUMARIO

La Función Córticosuprarrenal Durante la Terapéutica con Hidracida Isonicotinotínica por Tuberculosis Pulmonar

La naturaleza de la respuesta del huésped durante la isoniacidoterapia por tuberculosis pulmonar indicaba un posible factor córticosuprarrenal. Con mira a determinar mejor esa posibilidad, se emprendió la observación de 8 tuberculosos pulmonares y el estudio detenido de 3 de ellos durante la isoniacidoterapia a dosis de 3 a 5 mg. diarios por kg. de peso durante doce a veinte semanas.

Todos los enfermos revelaron mejoría subjetiva observable en su actitud, y otros efectos anespecíficos junto con mayor o menor mejoría objetiva.

Utilizando las cifras de eosinófilos y los patrones de excreción de 17-quetosteroides, ácido úrico, creatinina y 17-hidroxicorticoides, se determinó el índice de actividad suprarrenal antes de la isoniacidoterapia y durante ésta. Se valoró la respuesta córticosuprarrenal por la prueba de la córticotropina intravenosa durante ocho horas antes de la terapéutica y durante ésta. En ningún caso, se observó ni en estado de reposo ni de excitación ninguna desviación importante o constante de lo normal.

Hubo un aumento gradual de los eosinófilos circulantes durante la isoniacidoterapia. La proporción de ácido úrico-creatinina urinarios varió en razón directa al proceso morboso, y se sugiere el uso ulterior de este procedimiento como índice de la actividad de la enfermedad.

Discútense las posibles importancia y limitaciones del estudio actual.

RESUME

La fonction cortico-surrénale au cours du traitement de la tuberculose pulmonaire par l'hydrazide de l'acide isonicotinique

La nature de la réaction du sujet au cours de l'isoniazide-thérapie pour tuberculose pulmonaire suggérait la possibilité d'une influence cortico-surrénale. Il a été procédé à l'observation de 8 malades atteints de tuberculose pulmonaire et à l'étude extensive de 3 d'entre eux durant le traitement par l'isoniazide à la dose de 3 à 5 mg par kg pendant des périodes de douze à vingt semaines pour obtenir des données confirmant cette possibilité.

Une amélioration subjective du psychisme a été observée chez tous les malades, en même temps que d'autres réactions non spécifiques et différents degrés d'amélioration fonctionnelle.

Le degré d'activité surrénale a été déterminé avant et au cours du traitement par l'INH par l'étude de l'éosinophilie, des 17-cétostéroides, de l'acide urique, de la créatinine et des modali tés de l'excrétion des 17-hydroxycorticoides. La réponse cortico-surrénale a été évaluée par le test de huit heures à la corticotrophine intraveineuse (eight-hour intravenous corticotropin test) avant et pendant le traitement. Au cours de la phase de repos ou de stimulation, il n'a été observé d'écart significatif ou constant par rapport à la normale dans aucun des cas.

Une élévation progressive des éosinophiles circulants s'est produite pendant la thérapie par l'isoniazide. La variation du rapport urinaire acide urique-créatinine était proportionnelle au processus de l'affection et l'auteur propose l'utilisation de ce phénomène comme indice du degré d'activité de l'affection.

La signification et les limitations possibles de l'étude actuelle sont commentées.

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STUDIES ON ISONIAZID AND TUBERCLE BACILLI[1, 2]

III. The Isolation, Drug-Susceptibility, and Catalase-Testing of Tubercle Bacilli from Isoniazid-Treated Patients

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INTRODUCTION

Difficulties have been encountered in isolating and maintaining subcultures of tubercle bacilli from isoniazid-treated tuberculous patients (1–3).

Previous studies from this laboratory (4, 5) have shown that certain isoniazid-resistant mutants isolated *in vitro* from stock laboratory strains of tubercle bacilli have special growth requirements for catalase or agents having catalase-like activity. The fact that such strains are highly attenuated for guinea pigs made it imperative that reliable methods be devised for isolating such strains from isoniazid-treated patients and maintaining them in artificial media. The principal purpose of this report is to evaluate the usefulness of an oleic-acid–albumin solid agar medium for the primary isolation of such strains from pathologic materials. Furthermore, it has been indicated (5) that catalase-positive, isoniazid-resistant mutants also may be isolated from isoniazid-treated patients. It will be shown that the daily dosage of isoniazid during the initial period of treatment with this drug can significantly influence the comparative incidence of the catalase-negative and catalase-positive resistant mutants which may later appear in sputum.

During the course of these studies, certain heretofore unrecognized growth requirements of tubercle bacilli have been revealed. These are apparently unrelated to the special requirements of catalase-negative, isoniazid-resistant mutants. However, it will also be a purpose of this report to present an account of these observations because of the advantages of primary isolation, drug-susceptibility, and catalase-testing of tubercle bacilli on a well-defined, transparent, flat agar medium.

MATERIALS AND METHODS

Media: The basic solid agar (1.5 per cent) medium used in these studies has been described in a separate communication (4). To this basic medium, containing 0.5 per cent oleic-acid–albumin (OA) complex, at pH 6.6, glycerol (reagent grade) was added before autoclaving and glucose, after autoclaving, in final concentrations of 0.2 per cent, and iron, proved to be stimulating under these conditions for isoniazid-resistant, catalase-negative strains of tubercle bacilli (4), was added before autoclaving in the form of ferric ammonium citrate at 0.1 gm. per liter. This medium was identified in the present laboratory as the "complete" 7 H-3 OA-agar medium. For the study of individual components of the medium,

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[1] From the National Jewish Hospital at Denver, Colorado, and the University of Colorado School of Medicine.

[2] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

such substances were either omitted and then added as specifically indicated under *Observations*, or were simply added to the OA medium previously described (6). Felsen quadrant "plates" (9) (*see* figure 2) were used as containers for the OA media, each quadrant containing approximately 6 ml. of solid medium. These plates were stored at room temperature and used within a few days in order to avoid the destruction of isoniazid, which is known to occur at 37°C. (10, 11); the half-life of isoniazid, as tested by microbiologic methods in this OA (or Tween®-albumin) medium in this laboratory, is approximately seven days at 37°C. and much longer than this at room temperature or at 4°C. After inoculation, the Felsen plates were incubated in closed plastic bags[3], each large enough to contain four to eight such plates; this precaution prevented excessive drying of the medium during incubation.

Early in the course of the studies a special investigation was made of the possible advantage of incubating the OA-type solid agar medium longer than twenty-one days in terms of the incidence of strains giving invisible growth (low-power microscopic observation of the agar surface) at twenty-one days but ultimately (at six to eight weeks) yielding detectable growth. The incidence of this phenomenon proved to be so low (less than 1 per cent of all specimens positive on smear or on culture on American Trudeau Society (ATS) medium, or both) that, since then, Felsen plate cultures which are negative on microscopic examination of the agar surface at twenty-one days have been routinely discarded.

Culture tubes of the ATS medium, on the other hand, were kept under incubation until detectable growth occurred, or for at least eight weeks after inoculation. Longer incubation than eight weeks seemed unnecessary in view of the very low incidence of sputum cultures which are positive only after this period (12).

Certain of the analytical data reported here were obtained with the use of Petri dishes (instead of Felsen plates), each containing 20 to 25 ml. of medium. These were usually read at fourteen to eighteen and at twenty-one days to detect growth stimulation or inhibition by various substances on strains which grew well in twenty-one days.

Malachite green (Coleman and Bell Co.) at a concentration of 1 γ per ml. was added to all OA-agar media used for primary isolation; this concentration has proved, for this purpose, innocuous in several special studies which will not be described here. The concentration of malachite green, 3.2 γ per ml., employed by Frisk and Lagergren (13) was found in the present laboratory to be toxic in the OA-agar media for several strains of tubercle bacilli.

The American Trudeau Society (ATS) medium (14) was prepared as follows, and distributed in screw-capped tubes, 15 mm. by 15 cm.

Formula:

Egg yolk	400 ml.
Potato water (4 per cent glycerin)	400 ml.

(*a*) The potato water is made by grinding 100 gm. of pared potato in 400 ml. of water; enough glycerin is then added to give a final concentration of 4 per cent by volume, and the mixture is autoclaved in a two-liter flask for thirty minutes at 10 pounds of pressure. It is then cooled to 50°C., and the 400 ml. necessary for the formula are decanted.

(*b*) The egg yolk is prepared as follows: Fresh hens' eggs are carefully cleaned with wet gauze, then immersed in 70 per cent alcohol for fifteen minutes, and dried with sterile towels. The egg whites and yolks are separated in the usual manner. As much as 20 per cent of the egg white may be left with the yolks without deleterious effect on the medium.

(*c*) The 400 ml. of egg yolk is poured into the 400 ml. of potato water and to this is added 10 ml. of a 1 per cent aqueous solution of malachite green. All ingredients

[3] Available commercially from A. E. Macadam & Company, Inc., Brooklyn 38, New York (6″ x 3″ x 15″).

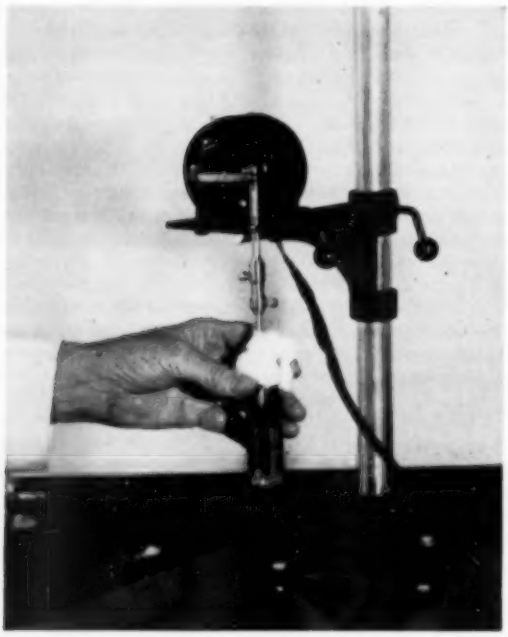

FIG. 1. "Tongue vibrator" used to homogenize sputum with solution of digesting material.

are thoroughly mixed, then filtered through sterile gauze. The pH will be approximately 7. The medium is then tubed, slanted, coagulated, and sterilized by a single one-hour stay in an inspissator heated to 90°C.

Directions for collection, digestion, decontamination, and inoculation of pathologic materials on artificial media:

1. Collect up to, but not exceeding, 10 ml. of sputum during a period of no more than seventy-two hours directly into a cellulose nitrate tube capped with a cork stopper. The tube should be labeled with a tape on which is written the patient's full name. The tube should be supported in a glass bottle.

   N.B. If storage is necessary after collection, it should be in the cold.
2. To each sample of sputum collected, add 1.0 ml. of a solution of "activated"[4] Difco Pangestin® prepared as follows: Dissolve-suspend 1 part of Pangestin® in 25 parts of distilled water; centrifuge at high speed in the angle centrifuge in the cold; pour off the clear, amber-colored supernate. Mix this solution with an equal volume of a 12 per cent aqueous solution of magnesium sulfate. Store in the deep-freeze cabinet in approximately 17 ml. amounts in each 20 ml. lusteroid tube.
3. Add 1.0 ml. of borate buffer (10 per cent sodium borate at pH 8.0), and make up to final volume of 15 to 20 ml. with distilled water.
4. Under a ventilated hood with draft in operation, disperse this mixture by means of a "tongue vibrator" (figure 1) for a period of two minutes, shielding the top of

[4] "Activated" in the sense that magnesium increases the activity of desoxyribonuclease (15).

the tube with cotton (use a fresh cotton shield with each specimen, as well as a fresh stainless steel tongue).

5. Incubate at 40°C. in a water bath for thirty minutes.
6. Add another 1.0 ml. of "activated" Pangestin®.
7. Incubate at 40°C. for another thirty minutes.
8. Add distilled water to a final volume of 40 to 45 ml.
9. Centrifuge at 10,000 r.p.m. (or 3,000 r.p.m.) for ten minutes.
10. Pour off the supernate into a solution of disinfectant (*see* step 16, below).
11. Suspend the sediment in approximately 40 ml. of solution containing 0.5N sodium hydroxide and 0.05M trisodium phosphate.
12. Incubate at 40°C. for thirty minutes.
13. Centrifuge at 10,000 r.p.m. (or 3,000 r.p.m.) for ten minutes.
14. Immediately discard supernate.
15. Immediately suspend sediment with sterile capillary pipette in approximately 1 ml. of the following sterile solution: Equal volumes of 5 per cent sterile albumin and distilled water contaning 0.5 ml. of 1 per cent brom thymol blue, and containing 1 ml. of a 0.1 per cent solution of lima bean trypsin inhibitor[5] per 100 ml. Add 1N hydrochloric acid dropwise to adjust suspension approximately to neutrality.
16. Just before step 11, remove a portion of sediment to a glass slide for smearing and Ziehl-Neelsen staining for acid-fast rods. During the subsequent steps, examine microscopically for tubercle bacilli. Divide the specimens accordingly into three groups:
    I. Containing no, or less than 20, acid-fast rods in 20 oil-immersion fields.
    II. Containing 1–10 acid-fast rods per field.
    III. Containing more than 10 acid-fast rods per field.
17. For specimens of Class I, inoculate directly each of the four quadrants of one Felsen plate with equal volumes (0.1 to 0.2 ml.) of the undiluted suspension of the concentrated material. These quadrants contain drugs as follows:
    I. No drug (but, containing 5 γ of congo red per ml., autoclaved with the medium).
    II. 10 γ of streptomycin per ml. (added aseptically after autoclaving).
    III. 1 γ of isoniazid per ml. (autoclaved with the medium).
    IV. 10 γ of isoniazid per ml. (autoclaved with the medium).
18. For specimens of Class II, add approximately 9 ml. of sterile distilled water and plate out this dilution of the suspension in the same fashion (10^{-1} dilution).
19. For specimens of Class III, dilute to 10^{-2} in 0.2 per cent sterile albumin in sterile distilled water and plate out in the same fashion.
20. In addition, make tenfold higher dilution of the suspensions of concentrated material than are indicated in 17, 18, and 19, above, and inoculate a second Felsen plate with this tenfold diluted material.
21. Inoculate two tubes of ATS medium containing no antimycobacterial drugs with the same volumes of concentrated material as are used for the inoculation of each quadrant of the Felsen plates; one tube, with the dilution of material indicated according to 17, 18, and 19, above, and the other tube with the tenfold higher diluted material.

Procedure for gastric contents: The procedure for isolating tubercle bacilli from freshly collected gastric contents is the same as that described above for sputum, except:

(*a*) To the sample of gastric contents, add 0.1 volume of borate buffer at pH 8.0 (the material may be stored at this point in the ice box for a few days).

(*b*) Add 0.1 volume of the "activated" Pangestin®, and

[5] Obtainable from Worthington Biochemical Corp., Freehold, N. J., and employed to assist in preventing the pancreatic trypsin carried over from digesting the albumin in the medium.

(*c*) Carry on from step 4 as with sputum.

Procedure for urine:

(*a*) Add 1N sodium hydroxide with stirring to obtain flocculation; if no flocculation occurs, add approximately 5 ml. of 10 per cent calcium phosphate to get flocculation.

(*b*) Transfer to a tall cylinder of appropriate size.

(*c*) Allow floc to settle out to total volume of less than 100 ml. (Material may be allowed to stand indefinitely in refrigerator at this point.)

(*d*) Pour off, or suck off, supernate with a sterile pipette (for each specimen) attached to rubber tubing and a water-pump suction apparatus.

(*e*) Transfer sediment to glass 50 ml. centrifuge tubes; then spin, full speed, in ordinary centrifuge, and decant.

(*f*) Resuspend total sediment in 0.1N hydrochloric acid at a final volume of approximately 40 ml.; then transfer to a cellulose nitrate tube.

(*g*) Carry on at this point, together with gastric washings and sputa, as in step 11 of sputum procedure, above.

* * *

Catalase tests: Catalase tests on colonies of acid-fast organisms were performed as previously described (8). The results of such tests permitted their classification in the following categories:

Positive (++ or +++), if the evolution of bubbles was clear-cut within two or three minutes, at the longest, after exposure to the test solution containing 5 per cent Tween® 80 and 15 per cent peroxide. (The strongest catalase reaction given by any strain of tubercle bacillus was recorded as a 3-plus reaction, although weaker reactions were often given by isoniazid-susceptible strains when colonies less than 1 mm. in diameter were tested.)

Doubtful (+), if only a weak reaction was given by a colony 1 mm. or larger in diameter, or if the available colonies were too small to give a reliable test.

Negative (− or ±), if no significant reaction was observed.

Mixed, if colonies on the control, on the streptomycin quadrant, or on both, gave positive reactions, while the colonies on the quadrants containing 1 γ or more of isoniazid per ml. were negative in the test. This reaction is given by ordinary mixed populations of bacilli, some susceptible and others resistant to 1 γ of isoniazid per ml.

"*Special mixed*," if approximately equal numbers of colonies grew out on the quadrant containing no isoniazid and on the quadrant containing 1 γ of isoniazid per ml., and positive reactions were given by any colonies on quadrants containing isoniazid. This result indicates the presence of isoniazid-resistant (resistant to 1 γ of isoniazid per ml.), catalase-positive mutants.

Effect of size of inoculum of suspension of sputum concentrate material on the subsequent growth of tubercle bacilli: Preliminary studies have shown that, when OA-type solid agar medium is inoculated in duplicate, both with the undiluted suspension of concentrated material and with tenfold dilutions of such, positive cultures are occasionally obtained only from the tenfold diluted inoculum. This phenomenon, by no means uncommon in the writers' experience, led to the two dilutions of inocula from each specimen employed in the clinical laboratory investigation described below.

This two-dilution method of inoculation also presents the advantage of permitting a more accurate estimate of the incidence of drug-susceptible organisms in populations containing many drug-resistant mutants. Significant proportions of drug-susceptible organisms may easily be obscured by too heavy inocula.

Furthermore, the washing and planting of more than one dilution of suspensions of pathologic materials have been found to be important for the interpretation of the results of catalase tests on colonies of mycobacteria isolated therefrom. On several occasions in this laboratory, falsely positive catalase tests have been encountered; these were attributable to the catalase of tissue materials carried in the inoculum and manifesting activity

even after three or more weeks of incubation on the culture medium. This phenomenon occurs with regularity with undiluted inocula of suspensions of tissues or cavity contents on the 7 H-3 medium, and may be avoided by dilutions of such materials when catalase tests are to be performed.

OBSERVATIONS

Formulation of the Routine Clinical Laboratory Agar Medium

Strains inhibited by asparagine: At least two strains, M. D., No. 11085[6] (beta-variant), and M. T., No. 11407, have been observed repeatedly to be incapable of multiplying on the OA-agar medium containing 0.05 to 0.2 per cent asparagine. These strains grew to yield grossly visible colonies at fourteen to twenty-one days on the medium containing 0.05 to 0.1 per cent malic acid or glutamic acid (as sodium salts) in place of asparagine. The addition of asparagine at 0.1 per cent concentration inhibited growth in the presence of 0.05 to 0.1 per cent malic acid. Although sodium glutamate did not permit quite so rapid growth of these strains as did sodium malate, glutamic acid at 0.05 per cent concentration has not been observed to inhibit any strain of tubercle bacillus thus far encountered. One strain, Vallée, commonly used as a stock laboratory strain for experimental studies, is somewhat inhibited by as little as 0.02 per cent sodium malate on the OA-type agar medium.

Indication of simultaneous presence of two strains of Mycobacterium tuberculosis: Especially striking is the fact that each sputum specimen of patient M. D., No. 11085, contained, during a period of several months of this study (long after initiation of therapy with isoniazid), at least two different strains of tubercle bacilli. Both were resistant to 10 γ of isoniazid per ml. of medium and highly attenuated for guinea pigs. However, one strain was inhibited by asparagine (beta-variant), and the other not (alpha-variant). It is noteworthy that, during the period when these specimens were received for study, the patient's chest roentgenogram revealed two, and probably more, open cavities.

Inhibition by amino acid mixtures: It was previously recommended (6) that final concentrations of 0.1 to 0.2 per cent of an enzymatic digest of casein be used in synthetic media for the cultivation of tubercle bacilli. Extensive clinical laboratory studies have shown that a concentration of 0.1 per cent of such material is inhibitory for several strains of tubercle bacilli in the OA-type media. The writers have also observed that some strains are slightly inhibited by 0.05 per cent concentration of such material. The vitamin-free casein (acid-hydrolyzed and extracted, from Nutritional Biochemicals Corp., Cleveland 28, Ohio) used subsequently in these investigations for the explicit purpose of discovering "vitamin" requirements of tubercle bacilli is similarly toxic at 0.05 per cent final concentration for some strains. Indeed, although in the experiments described here the low, nontoxic, concentration of 0.01 per cent has been employed, it should be pointed out that no evidence has been adduced in the present laboratory that any strains of tubercle bacilli, even on primary isolation, manifest requirements for the amino acids contained in such preparations.

These observations of the toxicity of the high concentrations of such amino

[6] National Jewish Hospital clinical record numbers.

acid mixtures may be related to Dubos' observation that DL-serine and DL-alanine are inhibitory for some strains of virulent tubercle bacilli (16).

Requirements for sulfate ion: Two sputum concentrates, one from Patient J. Z., No. 10722, and the other from Patient L. G., No. 9867, were planted on the complete 7 H-3 medium modified as follows: ammonium chloride was substituted for the ammonium sulfate, and magnesium sulfate was reduced to the previously recommended (7) concentration of 0.01 gm. per liter. Only a few very small, but grossly visible, colonies were obtained after twenty-one days of incubation of sputum concentrates from these patients on this medium. However, addition of 0.02 gm. of sodium sulfate per liter to this medium permitted many large colonies to grow from the same sputum specimens in the same period of time. This experiment was repeated with additional specimens from these same patients, with the same results. It was considered possible that a thiamine deficiency might be responsible for this peculiar requirement for sulfur. Thiamine, as a vitamin (1 to 10 γ per ml.), did not abolish the requirement of these strains for added sulfate ions in the OA-agar medium. The requirement of tubercle bacilli for sulfate ion has been described previously by Corper (17).

Requirements for biotin: The requirements of several strains for this vitamin, or for "physiological" concentrations of carbon dioxide will be described in detail elsewhere (18). Suffice it to state here that, unless cultures from a significant percentage of pathologic specimens from human beings are incubated on the OA-type agar media in an atmosphere containing a higher partial pressure of carbon dioxide than is available in ordinary air, poor growth or no detectable growth will occur without the addition of biotin in concentrations of 0.1 to 0.5 γ per ml. of medium.

Requirement for pyridoxine: This member of the B complex of vitamins has definitely, but irregularly, improved the growth rates of many different strains of tubercle bacilli on OA-type agar media. Unlike the situation with regard to biotin, no strains have been observed which manifest qualitative requirements for this factor. Nevertheless, in contrast to the completely negative results of studies with thiamine, riboflavin, folic acid, and whole yeast extract (except with one strain, to be described below) added to the 7 H-3 medium containing biotin and pyridoxine (or pyridoxal), the results with pyridoxine, it is believed, justify its inclusion in the 7 H-3 medium. It is obvious, however, that further studies on this factor are required.

Requirement for a factor in yeast extract: One strain, from patient A. G., No. 10140, grows poorly in the 7 H-3 medium on subcultivation, as well as on primary isolation, without the addition of 0.1 to 0.5 per cent yeast extract (Difco) to that medium. The yeast extract can be filter-sterilized or added to the basal medium before autoclaving without detectable loss of activity in this respect. It is, therefore, concluded that this "A. G. factor" in yeast extract is heat-stable. Attempts to obtain equal stimulation of growth of this strain by addition of thiamine, more biotin, pantothenic acid, more pyridoxine, folic acid, vitamin B_{12}, more fatty acid (oleic), whole bovine serum, more sulfate, magnesium, phosphate, iron or hemin, pyruvate, catalase, cobalt, manganese, more calcium, zinc or copper, and purines and pyrimidines have uniformly failed.

It will be noted that the medium (7 H-3, complete) employed in the practical clinical laboratory survey described below does not contain yeast extract. There were two reasons for omitting it: first, yeast extract at as low as 0.1 per cent final concentration was in previous studies seen to decrease the growth rates (in terms of colony size at the twenty-first day of incubation) of a significant number of strains of tubercle bacilli on primary isolation; and, second, only one strain has thus far been identified, among more than 300 different strains studied, as requiring yeast extract.

Effects of pyruvic acid: The addition of pyruvic acid (as the sodium salt) to the 7 H-3 medium in a final concentration of 0.02 per cent before or after autoclaving the basal medium has special effects on growth of many strains of tubercle bacilli. Some few strains are slightly inhibited, while others, especially isonizaid-resistant strains, are definitely stimulated even though the complete medium also contains 0.1 gm. of ferric ammonium citrate per liter. In separate communications (4, 5) this concentration of inorganic iron has been shown to favor growth of these more fastidious strains[7]. In reality, the omission of pyruvate from the medium described here is directly attributable to the fact that pyruvic acid is antagonistic to the antimycobacterial activity of isoniazid, a phenomenon previously reported (19) and fully confirmed in these studies. Thus, unless special provisions and qualifications are made, the use of a medium containing as little as 0.02 per cent pyruvate would interfere with "direct," quantitative, isoniazid-resistance tests on primary isolation.

Gross carbon sources (glucose, glycerol, oleic acid): Much investigation has been devoted in these studies to the determinations of optimal concentrations of glucose, glycerol, and oleic acid, alone and in various combinations, required for the primary isolation of tubercle bacilli from pathologic materials. A detailed description of these studies would be too extensive for the present report. Suffice it to state that the concentrations used in these studies, with the 7 H-3 medium, 0.2 per cent glucose, 0.2 per cent glycerol, and the 0.005 per cent oleic acid (final concentration) from the OA complex are near the optimal proportions for growth in the 7 H-3 OA agar medium. Higher concentrations of glucose, glycerol, or oleic acid are toxic for some strains, stimulatory for others. The addition of glycerol, even in only 0.2 per cent final concentration, has a marked stimulatory effect on the growth of nearly all strains encountered. Higher concentrations, up to, but certainly not exceeding, 0.75 per cent (20), probably deserve further study.

Practical Clinical Laboratory Isolation of Tubercle Bacilli from Pathologic Materials

During the month of February, 1954, 428 specimens (No. 5739 to No. 6166) from 319 different patients were examined for tubercle bacilli in the routine clinical laboratories. Three hundred and nine of these specimens were sputum;

[7] Growth stimulation by pyruvate has been observed especially with sputum specimens from tuberculous patients shortly after initiation of therapy with isoniazid, an observation which is particularly noteworthy in view of the stimulating effect of pyruvate on the growth of isoniazid-resistant mutants on primary isolation from parent-susceptible populations *in vitro* (7).

91 were gastric washings; 13 were urines; 15 were cerebrospinal fluids, pleural fluids, bronchial washings, or pus. All of these specimens were submitted, as described above under *Materials and Methods*, to digestion, decontamination, concentration, study of the stained smear, and simultaneous inoculation at two different dilutions on two Felsen plates containing the complete 7H-3 OA medium and on two tubes of ATS egg-yolk–glycerin–potato medium. The Felsen plate culture media were examined on the twenty-first day after inoculation and then discarded. The ATS culture media were examined at twenty-one days, four, five, six, seven, and eight weeks after inoculation.

Of the total number of specimens examined, 129 from 95 individual patients were either positive for acid-fast rods on microscopy, positive for *M. tuberculosis* on culture on either type of medium, or positive on both types of observation. In table 1 are presented the general results of this study.

Not included in table 1 are the following observations of some interest:

1. The total number of specimens which were positive with the tenfold diluted inoculum but negative with the undiluted inoculum on either medium was thirteen. This situation was no more commonly observed on the OA-agar than on the ATS medium.

2. The cultures on OA 7 H-3 medium from at least two of the specimens in this series, when studied microscopically with the low-power objective, were seen to consist of two types of colonies clearly distinguishable on the basis of size. One type was large and easily visible grossly; the other, very small and not clearly visualized without microscopic aid. These observations, alone, are sufficient to indicate

TABLE 1

RESULTS OF THE STUDY OF 129 SPECIMENS OF PATHOLOGIC MATERIALS POSITIVE FOR ACID-FAST RODS ON MICROSCOPY OR M. TUBERCULOSIS ON CULTURE ON EITHER OA 7 H-3 MEDIUM OR ATS MEDIUM

	Positive on Smear (93)				Negative on Smear, Positive on Culture (36)		
	Positive on OA and on ATS	Positive on OA Negative on ATS	Positive on ATS Negative on OA	Negative on Both Media	Positive on OA and on ATS	Positive on OA Negative on ATS	Positive on ATS Negative on OA
OA (3 weeks)	79	3*	—	11†	17	9 (9)‡	—
ATS (3 weeks)	54	—	0		7	—	5
(4 weeks)	22	—	0		7	—	2
(5 weeks)	3	—	0		3	—	1
(6–8 weeks)	0	—	0		0	—	2
Total	79		0		17		10 (10)‡

* One of these three cultures was negative on ATS, probably because of contamination on both tubes of this medium.

† See text for explanation of these negative cultures.

‡ Numbers in parentheses designate that in these instances so few colonies appeared as to indicate that differences between growths on different media should be attributed to chance distribution of very small numbers of bacilli in the inocula.

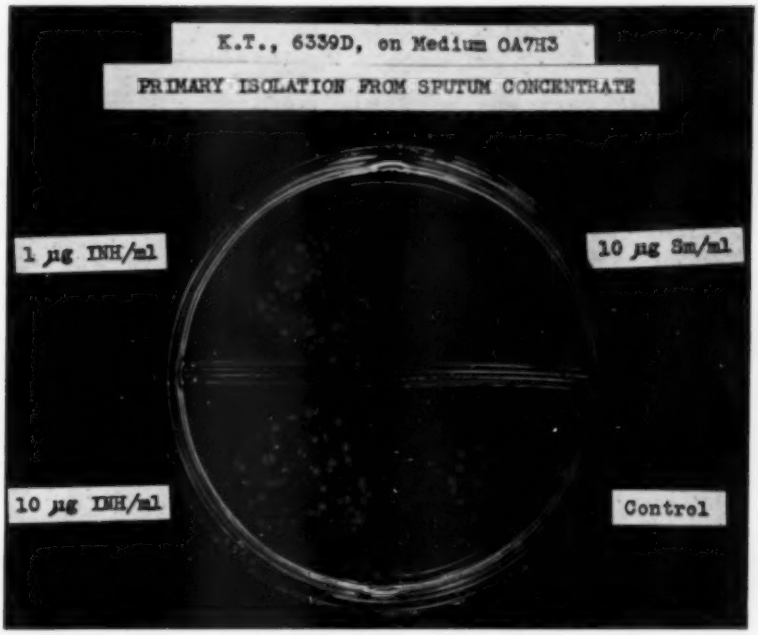

FIG. 2. Growth of colonies of tubercle bacilli on medium in Felsen plate. Note two different types of colonies and the apparent stimulation of one strain by isoniazid.

that, for some few remaining strains of tubercle bacilli, the complete OA 7 H-3 medium is at least somewhat deficient or contains a toxic excess of an unidentified component. It is also possible (*see* 5 below) that the environmental concentration of carbon dioxide in air is too low to permit rapid initiation of growth even in the presence of 0.5 γ of biotin per ml. for some strains.

3. On more than one occasion it has been observed in the routine clinical bacteriology laboratory that an isoniazid-resistant, catalase-negative strain of tubercle bacilli may be stimulated by isoniazid at 10 γ per ml. on primary isolation from sputum. Figure 2 shows the appearance presented in one such case. This sputum specimen contained two isoniazid-resistant mutants, one of which grew equally well on the OA 7 H-3 agar medium on the quadrant containing no isoniazid and on the quadrants containing 1 γ and 10 γ of this agent. The other strain was obviously stimulated somewhat by 1 γ and markedly by 10 γ of isoniazid per ml. Subculture of colonies of the "isoniazid-stimulated" strain into Tween®-albumin medium containing 10 γ of isoniazid per ml. and plating out on the 7 H-3 OA-agar medium with and without isoniazid failed to reproduce the apparent stimulatory effect of isoniazid. Experiences with other similar strains have been equally unsuccessful thus far in permitting the demonstration of a genotypic characteristic of such strains which could explain the evident facilitating effect of isoniazid on their growth directly from pathologic materials.

4. The comparative incidence of contamination of the OA 7 H-3 (total of 856

Felsen plates) and the ATS (total of 856 tubes) media were as follows: 11 Felsen plates of OA 7 H-3 medium (8, inoculated with the undiluted inoculum; 3, inoculated with the tenfold diluted material) manifested contamination sufficient to obscure examination of the medium for tubercle bacilli on the control quadrant—an incidence of 1.3 per cent. On the other hand, 55 tubes of ATS medium (42, inoculated with the undiluted material; 13, inoculated with the tenfold diluted material) were similarly contaminated—an incidence of 6.4 per cent. Furthermore, it is important to add that, although both tubes of ATS cultures from five specimens were contaminated, in no single case were both of the Felsen plates contaminated.

5. Eleven of the 129 specimens were positive for acid-fast rods on microscopy but negative for *M. tuberculosis* on culture.

One was a specimen of pus from a long-standing abscess of the neck of a child treated for several months previously with isoniazid and streptomycin.[8]

Two specimens of sputum which are worthy of special note were derived from patient M.D., No. 11224, whose bacilli have been the subject of investigation in this laboratory for more than sixteen months. This patient was admitted to the hospital after a previous period of therapy with isoniazid. Many attempts were made to cultivate on artificial media the acid-fast rods which have invariably appeared in enormous numbers in her sputum, some fifty specimens of which have been studied. In unsuccessful attempts the following substances were reduced in concentration in, or removed from, the 7 H-3 medium:

iron, magnesium, calcium, zinc, copper, biotin, pantothenic acid, pyridoxine, glucose, glycerin, oleic acid. Similarly, the following sources of nitrogen or "sparking" agents have been substituted for glutamic acid: asparagine, malic acid, and aspartic acid. The following substances were added, equally without success: hemin (10 and 100 γ per ml.), pyruvic acid (0.01 to 0.2 per cent), yeast extract (0.1 to 0.5 per cent), folic acid (1 γ per ml.), thiamine (1 to 10 γ per ml.), whole bovine serum, rabbit's blood, liver extracts. However, the 7 H-3 medium inoculated with the concentrated material from this patient's sputum and incubated under an atmosphere of 5 per cent carbon dioxide in air has, in additional experiments, repeatedly yielded good growth of tubercle bacilli.

Injections of sputum concentrates from this patient, No. 11224, into guinea pigs, subcutaneously, on several occasions have not even given rise to local abscesses at the sites of inoculation within seventy-two days after challenge. Nevertheless, exposure of ten mice to an aerosol prepared by grinding the sputum concentrate specimen from this patient in distilled water (*see* previous communication (21)) resulted in the development of three grossly visible tuberculous pulmonary lesions within thirty days after exposure. These lesions contained many acid-fast rods demonstrable on microscopic section. Attempts at subculturing the organisms from these lesions of the mouse lungs also failed, except when the inocula were made on the OA 7 H-3 medium and the culture was incubated under 5 per cent carbon dioxide in air.

These results indicate that for some strains of tubercle bacilli growth may not occur, even with added biotin, except under "physiological" concentrations of carbon dioxide. Further studies with carbon dioxide since this clinical laboratory study was completed suggest that most strains of tubercle bacilli from pathologic materials yield visible colonies much earlier on the complete 7 H-3 OA medium incubated under 5 per cent carbon dioxide in air than under ordinary atmospheric air plus biotin.

[8] It is a common experience in this laboratory, as in others, that closed necrotic lesions containing acid-fast rods are often negative on culture.

Of the remaining eight specimens which were positive on smear for acid-fast rods, but negative on culture, one was from urine and was probably *M. smegmatis*, and seven were from patients soon after they were initiated on therapy with isoniazid. The latter structures may have represented drug-sterilized tubercle bacilli.

Results of Catalase Tests

Catalase tests were performed on all cultures positive for *M. tuberculosis* in this series, except in those few cases in which the colonies were too small at twenty-one days to permit any valid conclusions to be drawn. Tests of the colonies on ATS medium which appeared after twenty-one days of incubation were performed as soon as sufficient growth had occurred.

In several cases, on ATS medium it was possible immediately to identify the growth as that of a contaminant by the excessive evolution of gas when the catalase test was performed. All colonies of tubercle bacilli which appeared on either of the two types of culture media employed in this study were less strongly catalase-positive than were the contaminants, including two acid-fast contaminants which on subculture grew rapidly at room temperature on the OA 7 H-3 medium and were less acid-fast than tubercle bacilli. There was one exception to this generalization. A strain of atypical tubercle bacilli was repeatedly isolated from patient H. B., No. 11576 which was similar to the strains of "yellow bacillus" described by Tarshis and Frisch (22) and by Buhler and Pollak (23). This strain gave rise to colonies which were strongly positive in the catalase test. The details concerning this strain will be described elsewhere.

The general results of the catalase tests performed in connection with this limited study and on cultures from additional patients are recorded in table 2.

With regard to the results of catalase tests of colonies of *M. tuberculosis* on

TABLE 2

RESULTS OF CATALASE TESTS CORRELATED WITH ISONIAZID-RESISTANCE TESTS ON PRIMARY ISOLATION OF TUBERCLE BACILLI FROM PATHOLOGIC MATERIALS

Positive Cultures from at Least One Specimen, Each, of 121 Different Patients Studied

	"Catalase-Positive"*	"Catalase-Negative"	"Mixed"	"Special Mixed"
Growth on OA 7 H-3				
Isoniazid-susceptible†	53† (12)‡	0	—	—
Isoniazid-resistant§	—	41 (15)	—	20 (0)
Mixed populations	—	—	7 (3)	—

* See *Material and Methods* for definitions.

† Susceptible to 1γ of isoniazid per ml. of OA 7 H-3 medium. Thirty-five of these patients had received no isoniazid therapy before the tested cultures were obtained for these studies; the remaining 18 specimens (patients) either were from patients who had been on isoniazid therapy for only a few weeks or the numbers of colonies isolated were very small.

‡ The numbers recorded in parentheses indicate the numbers of patients who had been started on therapy with 8 mg. of isoniazid per kg. per day.

§ Resistant to at least 1γ of isoniazid per ml., as indicated by apparently equal numbers of colonies on the control quadrants and on quadrants containing 1γ of isoniazid per ml.

ATS medium, it is sufficient to state that there was perfect correlation with the results obtained with colonies from the same specimens on the OA 7 H-3 medium containing no drug. In those few cases in which catalase tests were applicable only to colonies of mycobacteria on ATS medium, because of failure to obtain growth on the modified OA medium, no negative test results were obtained on growth from specimens of patients not previously treated with isoniazid.

All strains resistant to streptomycin and susceptible to 1 γ of isoniazid per ml. proved to be catalase-positive in this series.

Worthy of special attention are the twenty cases which gave results classified as "special mixed." Only four such cultures have been studied in any detail in this laboratory, two of which have been previously reported (15). Such isoniazid-resistant, catalase-positive, guinea pig-pathogenic cultures, nearly all of which have proved to consist of clearly definable mixtures of catalase-negative and catalase-positive, isoniazid-resistant mutants, have thus far appeared in the excreta only of patients initiated on treatment with isoniazid in dosage less than 8 mg. per kg. per day. They have been described in greater detail in a separate communication from this laboratory (5).

DISCUSSION

No claims can be made for superiority of the enzymatic digestion and decontamination method described here. It may be noted, however, that the enzymatic method (without alkali treatment) may be applied to the isolation of other microorganisms from sputum which otherwise would be so viscous that quantitative studies would be well nigh impossible. Furthermore, the use of a small amount of trisodium phosphate with the sodium hydroxide permits a better control of the pH of the digestion mixture, essentially uninfluenced by the bulk of the undigested sputum sediment. Moreover, the two steps of centrifugation, although somewhat more time-consuming, avoid the danger already pointed out (24) of carrying over orally administered antimicrobial agents (PAS or isoniazid) which might inhibit the growth of tubercle bacilli on the culture medium. In any event, the incidence in this series of positive cultures from concentrates negative for acid-fast rods on microscopy is sufficient evidence that these methods of digestion, decontamination, and inoculation are not inferior to the classical method which employs sodium hydroxide for both digestion and decontamination.

There is no need to elaborate on the comparative merits of the complete 7 H-3 oleic-acid–albumin solid agar medium and the ATS medium. It is probable that further improvements of this agar medium will be seen. Nevertheless, in terms both of the length of the period of incubation required and of the total number of positive cultures obtainable from this series of specimens, the OA 7 H-3 medium is clearly superior to the ATS medium under the conditions prevailing in the present laboratory. Furthermore, the flat surface of the agar on the Felsen plate permits, with much greater ease of handling, more or less quantitative estimations of the actual numbers of colonies growing on different quadrants in direct drug-susceptibility tests: the preparation of ATS and similar media

requiring inspissation in Felsen quadrant dishes has been practiced in this laboratory but is not recommended. The OA 7 H-3 and the ATS media are approximately equal in cost for the reason that the bovine serum albumin is so expensive. A substitute for this agent has been sought but not yet found. Finally, the identification of the striking differences in the growth requirements of different strains of tubercle bacilli were not recognized and, indeed, would probably not have been possible with the use of complex egg and potato or blood-agar media. For example, it is certainly clear that a requirement for biotin, which is in such high concentration in egg yolk and variably antagonized by the avidin present in egg white, could not have been recognized in such complex media.

Iron at a concentration of 0.1 gm. of ferric ammonium citrate per liter has been described elsewhere (4) as favoring the growth of catalase-negative, isoniazid-resistant mutants of tubercle bacilli. Those experiments were carried out *in vitro* on primary isolation on the OA 7 H-3 medium from parent drug-susceptible populations without antagonizing the antibacterial activity of isoniazid. It is clearly shown by these studies to be useful in the same medium for the isolation of such strains from isoniazid-treated patients[9].

The "complete" OA 7 H-3 agar and the ATS media, incubated under ordinary atmospheric air, both failed to yield growth of tubercle bacilli from seven or eight specimens which were positive for acid-fast rods on microscopy, in addition to the two specimens from patient No. 11224 which contained tubercle bacilli requiring added carbon dioxide for growth. Inasmuch as seven of these specimens were from patients who had been started on isoniazid therapy within the previous few weeks, it seems fair to assume that the acid-fast rods might have been isoniazid-sterilized tubercle bacilli. However, it is also possible that the acid-fast rods were tubercle bacilli which, like the strain from patient No. 11224, require added carbon dioxide. No opportunity to study this possibility has been afforded in these specific cases because smears of subsequent specimens from these patients have been negative for acid-fast rods, and cultures under 5 per cent carbon dioxide in air were not performed routinely during the special study reported here.

In any event, it has been shown that higher concentrations of carbon dioxide than are available in ordinary air have a stimulatory effect on the initiation of growth of many strains of tubercle bacilli from pathologic materials. Indeed, higher carbon dioxide tension is *essential* for the initiation of growth of at least one strain, even in the presence of added biotin. These observations suggest that an atmosphere containing a "physiological" concentration (1 to 5 per cent) of

[9] Since the preparation of the original manuscript of this report, it has been found possible to modify the OA 7 H-3 agar medium so as to favor even more strikingly the growth of catalase-negative, isoniazid-resistant tubercle bacilli. This modification has consisted of:

1. Addition of beef liver catalase, aseptically, at a final concentration of 2 γ per ml.
2. Reduction of ferric ammonium citrate to 0.02 gm. per liter.

This modification has been identified as the "7 H-7" medium.

The addition of this quantity of catalase does not detectably change the antimicrobial activity of isoniazid in the OA-agar medium; nor does it affect the results of catalase test on colonies grown on this medium.

this gas may profitably be employed for practical clinical laboratory isolation and cultivation of tubercle bacilli.

Failure of a streptomycin-resistant strain of tubercle bacilli to utilize asparagine has been independently observed and previously reported (25). Actual toxicity of this nitrogen source and "sparking agent" has not previously been reported except when concentrations in excess of 0.1 per cent were used (26). The presence in the same specimen of sputum of at least two strains of *M. tuberculosis*, distinguishable by virtue of their differing growth requirements, is not unique. This phenomenon has been observed not infrequently in sputum from other patients during the course of the present studies.

The general biologic implications of these studies are that wild-type tubercle bacilli are genotypically heterogeneous with respect to their growth requirements and that this heterogenicity may be increased by antimicrobial chemotherapy of disease "caused" by these agents.

The phenomenon of apparent stimulation of growth of isoniazid-resistant tubercle bacilli by isoniazid is unexplained. It may be suggested that isoniazid antagonizes in some fashion the bacteriostatic effect of a substance in the suspension of sputum concentrate material which may be inhibitory for some strains of tubercle bacilli. No proof of this hypothesis can be offered here.

It should be pointed out that the use of Petri dishes containing the OA 7 H-3 medium might be preferred to the Felsen quadrant plates for general use in laboratories primarily devoted to the bacteriologic diagnosis of tuberculosis. However, no elaboration is necessary on the advantages of the quadrant plates for laboratories required to do routine drug-susceptibility studies during or after courses of chemotherapy on known tuberculous patients. The simultaneous performance of culture and drug-susceptibility tests in such circumstances may save much valuable time in determining the course of therapy.

The results of the catalase tests described in this report take on special significance when they are integrated with the previously published observations from this laboratory (4, 5).

It has been demonstrated (5) that the attenuation of isoniazid-resistant mutants of tubercle bacilli is directly related to their deficiency in catalase activity. Comparative animal species studies have suggested that tuberculin-type hypersensitivity may play a dominant role in determining the peculiarities of the host-parasite relationship in infections with catalase-deficient strains of tubercle bacilli. If this be so, then most human beings may well behave more like guinea pigs or rabbits than like mice toward catalase-deficient tubercle bacilli. Human beings have a marked propensity to acquire tuberculin hypersensitivity, more marked, indeed, than either guinea pigs or rabbits. In this connection, however, claims have been made (27, 28) that isoniazid-resistant tubercle bacilli may be agents of progressive tuberculous infections in human beings. Such may well be true in view of our observations on tuberculous patients who were initiated on therapy with less than 8 mg. of isoniazid per kg. per day and continuously treated with this drug. Many such patients discharge in the sputum catalase-positive, isoniazid-resistant tubercle bacilli which may be pathogenic for guinea

pigs. On the other hand, such strains usually (5) manifest a moderate, though definite, degree of attenuation for guinea pigs. Thus, bona fide endogenous extensions of tuberculous infection in patients taking isoniazid alone might, theoretically, be rare. For example, they may be expected to be relatively more rare in such patients than they are in tuberculous patients who harbor fully pathogenic, streptomycin-resistant strains and continue on therapy with streptomycin alone.

No unequivocal evidence is available that catalase-deficient strains of tubercle bacilli are attenuated for human beings. Nevertheless, in view of the observations in the present studies, it would seem the better part of conservative wisdom to initiate isoniazid therapy in tuberculous human beings with a daily dosage of no less than 8 mg. per kg. of body weight. The sputum of 18 patients initiated on therapy with isoniazid for the first three months of treatment at this dosage level contained isoniazid-resistant tubercle bacilli. None of these organisms were catalase-positive. In a comparable group of patients treated initially with lower doses of isoniazid, at least one-third of the persons who excreted isoniazid-resistant organisms excreted catalase-positive, resistant organisms. Subsequent increase of the daily dosage of isoniazid in these patients to 8 to 10 mg. per kg. did not cause disappearance of the catalase-positive, resistant tubercle bacilli from the sputum.

The microbiologic explanation for this clinical-laboratory observation is suggested by the results of experiments described in the first paper of the present series of reports (4). Namely, it was seen that catalase-positive, isoniazid-resistant mutants may make their appearance on direct, primary isolation from a parent, drug-susceptible population *in vitro*. But they occur only in relatively small numbers and only in environments containing relatively low concentrations of isoniazid. Antimicrobially effective concentrations higher than these may usually be obtained in bacilli-laden, open, pulmonary cavities with daily doses of 8 mg. of isoniazid per kg.[10]

SUMMARY

Methods have been described for the practical isolation of tubercle bacilli from isoniazid-treated patients as well as from patients not previously treated with this drug. These methods were based upon the following observations, not previously described:

Two strains of tubercle bacilli are inhibited by asparagine, the commonest source of nitrogen used for the cultivation of tubercle bacilli in artificial media. The substitution of glutamate for asparagine has favored the growth of these strains and of all other strains of tubercle bacilli encountered in these studies. Moreover, previously recommended concentrations of casein amino acids are also toxic for many strains.

[10] The presence of more highly resistant, catalase-positive strains in the sputum of some isoniazid-treated patients may be explained by step-wise mutation of originally weakly resistant, catalase-positive mutants to higher resistance, without complete loss of catalase activity.

Biotin has been shown to be required for the initiation of growth of several strains of tubercle bacilli from small inocula. This requirement for biotin can be replaced by carbon dioxide. At least one strain has been observed to require for detectable growth an atmosphere containing carbon dioxide in higher concentration than is present in ordinary air, even in the presence of added biotin.

The growth of several strains of tubercle bacilli is stimulated somewhat by pyridoxine, although no qualitative requirement for this growth factor has been observed in these studies.

One strain of tubercle bacillus has manifested a requirement for an as yet unidentified factor in crude yeast extract for optimal growth.

Comparative clinical laboratory study of a medium formulated on the basis of the above observations and of a typical egg-yolk–glycerin–potato medium has revealed the former to be superior to the latter in the following respects:

It lends itself readily to the employment of a flat surface for the quantitative enumeration of tubercle bacilli and the relative incidence of mutants resistant to different chemotherapeutic agents at different concentrations in direct drug-susceptibility tests on primary isolation.

Growth of tubercle bacilli could be detected earlier, and, in two instances, large numbers of colonies occurred only on the former medium, indicating that the latter medium was inadequate for these strains.

Identification of different strains of tubercle bacilli in the same sputum specimen was strikingly facilitated by use of the former medium.

The incidence of contamination was significantly lower for the former medium.

Application of a test for catalase activity of colonies of tubercle bacilli from a large number of specimens in this routine clinical laboratory study has extended a previous report, permitting the following conclusions:

1. All strains of tubercle bacilli isolated from patients not previously treated with isoniazid manifest catalase activity *in vitro*.

2. With the exception of one strain of "yellow bacillus," an atypical "tubercle bacillus" which may be an agent of "tuberculosis" in man, the catalase test facilitated the identification of saprophytic mycobacteria and of other contaminants on the artificial media employed.

3. Nearly all mutants of tubercle bacilli resistant to 10 γ of isoniazid per ml. of oleic-acid–albumin 7 H-3 medium are catalase-negative or too weakly positive to manifest activity in the test employed. Strains of this type only were detected in the sputum of tuberculous patients started on treatment with no less than 8 mg. of isoniazid per kg. per day.

4. Many specimens from patients initiated on treatment with less than 8 mg. of isoniazid per kg. per day contain catalase-positive strains (widely variable, quantitatively) which are resistant to 1 γ of isoniazid per ml. of oleic-acid–albumin 7 H-3 medium, but susceptible to 10 γ of isoniazid per ml. of the same medium.

The possibility has been suggested that catalase-positive, isoniazid-resistant

tubercle bacilli may be more pathogenic for human beings than catalase-negative strains. On this account, it is recommended that initiation of isoniazid treatment of tuberculosis in the human being be with no less than 8 mg. per kg. per day, aiming to avoid the emergence of catalase-positive, drug-resistant strains.

SUMARIO

Estudios de la Isoniacida y los Bacilos Tuberculosos: III. E. Aislamiento, Farmacosusceptibilidad y Comprobación con Catalasa de los Bacilos Tuberculosos Procedentes de Enfermos Tratados con Isoniacida

Descríbense aquí métodos practicos para el aislamiento de bacilos tuberculosos procedentes de enfermos tratados con isoniacida e igualmente de enfermos no tratados antes con dicha substancia. Esos métodos se basan en las siguientes observaciones, hasta ahora inéditas:

Dos cepas de bacilos tuberculosos han sido inhibidas por la asparagina. La substitucion de la asparagina por un glutamato ha favorecido el crecimiento de estas cepas y de todas las otras cepas de bacilos tuberculosos encontradas en estos estudios. Ademas, las concentraciones de caseina digerida, recomendadas anteriormente, han resultado toxicas para muchas cepas.

Se ha demostrado que la Biotina es necesaria para la iniciacion del desarrollo de varias cepas de bacilos tuberculosos provenientes de inoculaciones de pequeñas cantidades. El requerimiento de Biotina puede ser reemplazado por anhidrido carbonico. A lo menos una cepa ha requerido para su crecimiento una concentracion de anhidrido carbonico mayor que la que se encuertra en el aire atmosferico, aun en presencia de Biotina.

La proliferacion de varias cepas de bacilos tuberculosos se estimula ligeramente por la Piridoxina, aunque no ha podido observare en estos estudios una relacion cualitativa respecto a este factor de crecimiento.

Una cepa ha requerido un factor aun no identificado en el extracto de levadura para su desarrollo optimo.

Estudios comparativos de laboratorio de un medio creado en base de las observaciones supramencionadas y de un medio tipico—yema de huero—glicerina-papa, han demostrado la superioridad del primero de la siguiente manera:

Préstanse fácilmente para el empleo de una superficie plana para la enumeración cuantitativa de los bacilos tuberculosis y la relativa incidencia de mutantes resistentes a distintos agentes quimioterapéuticos en diversas cifras de concentración en pruebas directas de susceptibilidad en el aislamiento primario.

Puede descubrirse antes la proliferación de los bacilos tuberculosos, y, en dos casos, sólo se presentaron grandes números de colonias en el primer medio, indicando esto que el último medio era inadecuado para estas cepas.

La identificación de distintas cepas de bacilos tuberculosos en el mismo ejemplar de esputo se facilitó sobremanera con el uso del primer medio.

El numero de contaminación es fué significativamente menor con el primer medio.

La aplicación de esta prueba para la actividad catalásica de colonias de bacilos tuberculosos procedentes de un amplio material en este laboratorio clínico amplía una comunicación anterior y permite sacar las conclusiones siguientes:

1. Todas las cepas de bacilos tuberculosos aisladas de enfermos no tratados antes con isoniacida manifiestan actividad catalásica *in vitro*.

2. Con la excepción de una cepa de "bacilo amarillo," un "bacilo tuberculoso" atípico, que puede ser agente de la "tuberculosis" en el hombre, la prueba de la catalasa ha facilitado la identificación de micobacterias saprófitas y de otros gérmenes contaminantes en los medios artificiales empleados.

3. Casi todas las mutantes de bacilos tuberculosos resistentes a 10 γ de isoniacida por ml. de medio 7 H-3 de ácido oleico-albúmina son catalasa-negativos o tan débilmente

positivos que no pueden manifestar actividad en la prueba empleado. Cepas de este tipe sólo se encontraron en el esputo de tuberculosos cuyo tratamiento se inició con no menos de 8 mg. de isoniacida por kg. al día.

4. Muchos material proveniente de enfermos cuyo tratamiento se inició con menos de 8 mg. de isoniacida por kg. al día contienen cepas catalasa-positivas (sumamente variables, cuantitativamente), que son resistentes a 1 γ de isoniacida por ml. de medio 7 H-3 de ácido oleico-albúmina, pero susceptibles a 10 γ de isoniacida por ml. del mismo medio.

Se ha sugerido la posibilidad de que los bacilos tuberculosos catalasa-positivos, isoniacidorresistentes pueden ser más patógenos para los seres humanos que los catalasa-negativos. Por esta razón, se recomienda que se inicie la isoniacidoterapia de la tuberculosos en el hombre con no menos de 8 mg. por kg. al día, con mira a evitar la formación de cepas catalasa-positivas farmacorresistentes.

RESUME

Etude sur l'isoniazide et le bacille tuberculeux: III. Isolement, mesure de la sensibilité à la drogue et application du test de l'activité catalasique des bacilles tuberculeux isolés de malades traités par l'isoniazide

Description de méthodes pour l'isolement de bacilles tuberculeux provenant de malades traités par l'isoniazide ou non traités. Ces méthodes sont basées sur les observations suivantes: Deux souches étaient inhibées par l'asparagine alorsque l'acide glutamique favorisait leur croissance. La caséine digérée employée à des concentrations excédant 0,1% était toxique pour un grand nombre de souches. Plusieurs souches necessitaient la biotine comme facteur de croissance ou incubation dans une atmosphère d'air dont la teneur en anhydride carbonique était augmentée. Cette atmosphère seule permettait d'obtenir la culture d'une souche.

L'étude clinique comparative d'un milieu formulé en tenant compte des observations citées et d'un milieu classique à l'oeuf a montré que le premier milieu était meilleur pour les raisons suivantes:

Sa surface plate permet, à l'isolement initial, la détermination du nombre des colonies et de la proportion de mutants résistant à divers agents chimiothérapeutiques employés à des taux différents.

La croissance des bacilles tuberculeux est décelée plus tôt, et, à deux reprises, le développement de nombreuses colonies n'a été obtenu que sur le milieu premier à l'oeuf, ce qui a démontré que le milieu était inadéquat pour ces souches.

L'identification des différentes souches de bacille tuberculeux dans un même spécimen de crachat était extraordinairement facilitée par l'emploi du milieu premier.

L'incidence de la contamination était abaissée de facon significative avec le milieu premier.

L'application d'un test pour la mesure de l'activité de catalase dans les colonies de BK, provenant de nombreux spécimens prélevés à l'occasion de cette étude clinique comportant les examens de laboratoire usuels, est une extension d'un rapport antérieur et a permis les conclusions suivantes:

1. Toutes les souches de bacille tuberculeux isolées chez des sujets non encore traités par l'isoniazide montrent *in vitro* une activité de la catalase.

2. A l'exception d'une souche de "bacille jaune", un "bacille tuberculeux" atypique pouvant être un agent de la "tuberculose" chez l'homme, le test de la catalase facilite l'identification des mycobactéries saprophytes et des autres contaminants dans les milieux artificiels utilsés.

3. Presque tous les mutants des bacilles tuberculeux résistant à 10 γ d'isionazide par ml. du milieu 7 H-3 acide oléique-albumine sont catalase-négatifs ou trop faiblement positifs pour manifester une activité dans les conditions du test. Seules, des souches de ce type ont été décelées dans les crachats des tuberculeux traités avec une dose intiale d'isoniazide qui n'étiat pas inférieure à 8 mg/kg par jour.

4. De nombreux spécimens provenant de malades traités avec une dose initiale d'isoniazide inférieure à 8 mg/kg par jour, renfermaient des souches catalase-positives (quantitativement très variables) qui se montrent résistantes à 1 γ d'isoniazide par ml. du milieu 7 H-3 acide oléique-albumine, mais sensibles à 10 γ d'isoniazide par ml. du même milieu.

L'hypothèse que les bacilles tuberculeux catalase-positifs et isoniazide-résistants pourraient être plus pathogènes pour les humains que les souches catalase-négatives est formulée. C'est pour cette raison que, chez l'homme, l'auteur conseille d'instaurer le traitement de la tuberculose par l'isoniazide avec une dose non inférieure à 8 mg/kg par jour, pour éviter l'apparition de souches catalase-positives et résistantes au composé.

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THE EFFECT OF TIME AND TEMPERATURE ON ANTIGENIC POTENCY OF BCG VACCINE[1]

II. Storage at 20° and 37.5° C.

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INTRODUCTION

It has been known for some time that the wide range of variations in tuberculin allergy and local vaccination lesions resulting from similarly prepared and standardized batches of BCG may be attributed to the effect of time and temperature on the living number of organisms per unit volume of vaccine. In the first article in this series (1), it was demonstrated that freshly prepared BCG vaccine stored at 2° to 4°C. lost 17 per cent of its viable organisms after one week, 39 per cent after two weeks, 57 per cent after three weeks, and 67 per cent after four weeks. It was further noted that a direct correlation could be established between the numerical living population of BCG aggregates present in the vaccination inoculum and the production of tuberculin allergy, local vaccination lesions, and specific resistance against a challenge tuberculous infection in the guinea pig. It was recommended, therefore, that vaccine stored at 2° to 4°C. should preferably be used within two weeks after preparation in order to obtain maximum sensitization and protection.

These experimental findings seemed at variance with the clinical reports by Edwards and Gelting (2, 3). The latter investigators had failed to find any apparent reduction in the allergy-producing capacity of BCG stored at 2° to 4°C. for at least two and one-half months; at room temperature (20°C.) for one month; at 30°C. for one week; and at 37.5°C. for five days. On the basis of tuberculin allergy, they concluded that "there would thus seem to be no reason why most vaccines must be used within ten to fourteen days of preparation in order to obtain satisfactory allergy."

The present writer is in agreement with Nissen Meyer and Palmer (4) that tuberculin allergy cannot be used independently to measure immunity after BCG vaccination and that allergy should be interpreted in relation to the vaccine's ability to produce vaccination lesions and specific immunity by protection tests. As a consequence, the investigation of the effect of time and temperature on the antigenic potency of BCG vaccine after storage at 20° and 37.5°C. up to four weeks has been continued.

MATERIALS AND METHODS

One hundred and twenty tuberculin-negative normal guinea pigs weighing 382 plus-minus 31.18 gm. were divided into ten groups, each comprising twelve animals. The sensitizing

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and immunizing antigen was BCG, lot B-133, which was prepared by the standardized method used in this laboratory (5). The vaccine was administered to nine groups of animals as follows:

To *Group 1:* Freshly prepared
To *Group 2:* One-week-old BCG maintained at 20°C.
To *Group 3:* One-week-old BCG maintained at 37.5°C.
To *Group 4:* Two-week-old BCG maintained at 20°C.
To *Group 5:* Two-week-old BCG maintained at 37.5°C.
To *Group 6:* Three-week-old BCG maintained at 20°C.
To *Group 7:* Three-week-old BCG maintained at 37.5°C.
To *Group 8:* Four-week-old BCG maintained at 20°C.
To *Group 9:* Four-week-old BCG maintained at 37.5°C.
Group 10: Uninoculated controls

The respective vaccines were administered similarly to each animal in all but the control group as follows: *intracutaneously* on the shaved right side of the abdomen, 0.1, 0.01, 0.001, and 0.0001 mg. of BCG contained in 0.1 ml. of phosphate buffer solution pH 7.2; *transcutaneously* on the shaved left side of the abdomen, forty punctures made with automatic apparatus (6) through skin soaked with 20 mg. of vaccine per ml.; *subcutaneously* 10 mg. of vaccine injected in the left hind leg. In this manner it was possible to observe the local production of vaccination lesions by methods universally employed, while the large subcutaneous inoculation served the purpose of sensitization and immunization. The number of living BCG aggregates in each batch of vaccine was determined by serial tenfold dilutions, each seeded on five tubes of Löwenstein egg medium and in Dubos liquid medium. The colony counts were made after four and eight weeks of incubation at 37.5°C. of 1 mg. of vaccine per ml., controlled by smears of Dubos medium.

OBSERVATIONS

Viability of BCG: The reduction of living BCG aggregates proceeded more rapidly at 20°C. (47 per cent reduction after one week, and 99.8 per cent after four weeks) than during storage at 2° to 4°C. Few living organisms survived after only one week's storage at 37.5°C. (91.5 per cent reduction) (table 1).

Intracutaneous and transcutaneous skin lesions: In table 2 it may be seen that the average maximum diameters of vaccination lesions produced in three weeks with graded doses of variously stored lots of BCG were uniformly greater with fresh vaccine than with vaccine maintained at 20°C. for more than one week, and that BCG maintained at 37.5°C. beyond one week produced the smallest

TABLE 1

VIABILITY OF BCG VACCINE STORED AT 2° TO 4°, 20°, AND 37.5°C. UP TO FOUR WEEKS

Type of Vaccine	2° to 4°C.*		20°C.		37.5°C.	
	Colonies in 1 Mg. of BCG per Ml.	Per Cent Reduction	Colonies in 1 Mg. of BCG per Ml.	Per Cent Reduction	Colonies in 1 Mg. of BCG per Ml.	Per Cent Reduction
Fresh	54×10^6	—	47×10^6	—	47×10^6	—
1-week-old	45×10^6	16.7	25×10^6	46.8	4×10^6	91.5
2-week-old	33×10^6	38.9	9×10^6	80.8	25×10^5	94.7
3-week-old	23×10^6	57.4	15×10^5	96.8	27×10^4	99.4
4-week-old	18×10^6	66.4	11×10^4	99.8	25×10^2	99.9

* Reference (1).

TABLE 2

AVERAGE MAXIMUM DIAMETERS (MM.) OF SKIN LESIONS OR PAPULES THREE WEEKS AFTER INJECTION OF GRADED DOSES OF BCG STORED UP TO FOUR WEEKS AT 20° AND 37.5°C.

(Twelve Guinea Pigs in Each Group)

Dose of BCG	BCG Vaccine								
	Fresh	1-Week Old		2-Week-Old		3-Week-Old		4-Week-Old	
		20°C.	37.5°C.	20°C.	37.5°C.	20°C.	37.5°C.	20°C.	37.5°C.
mg.									
0.1	6.3	6.1	4.5*	4.2*	4.0*	4.0*	3.2*	4.0*	3.1*
0.01	4.8	4.8	3.6*	3.0*	2.9*	2.6*	2.0*	2.7*	2.0*
0.001	3.2	2.9	2.1*	1.3*	1.0*	1.0*	0.5*	1.0*	0.0
20 per ml. (multiple puncture)	2.8	2.8	2.5	2.1	1.5*	1.7*	1.5*	1.5*	1.3*

* Significantly different from fresh vaccine group at the $P \leq 0.01$ level. In Fisher's (7) tables for distribution of t (quotient expressing deviations as multiple of its probable error), it was observed that a $P \leq 0.01$ (probability that the observed mean deviation bearing this or smaller values cannot have occurred by chance alone) requires that $t \geq 2.819$ when each group contains 12 animals or samples.

vaccination lesions. The same relationships were noted in the size of multiple-puncture papules. When examined two months after inoculation, the skin lesions were still visibly raised and inflamed in all of the animals inoculated with fresh and 20°C. stored vaccine, while the inoculated sites were flattened and mostly healed in animals inoculated with 37.5°C. stored vaccine. The regional axillary and inguinal lymph nodes were palpably larger and firmer in animals inoculated with fresh and 20°C. stored vaccine than with 37.5°C. stored vaccine, which seemed to have passed the peak of enlargement after approximately six weeks.

Subcutaneous reaction to BCG: Two weeks after the subcutaneous inoculation with 10 mg. of BCG in the left hind leg, all of the animals presented marked tumefaction around the BCG focus, followed by uniform softening, fluctuation, and abscess formation. Whenever the abscess ruptured through the inoculation channel, prompt healing followed. No appreciable difference in hyperplasia of the left inguinal lymph nodes was noted up to three months after inoculation except in *Groups 7* and *9*, which had been inoculated with vaccine stored at 37.5°C. for three and four weeks, respectively. In these groups a definite regression was noted in lymphonodular hyperplasia two months after inoculation.

Tuberculin hypersensitivity: The degree of tuberculin allergy elicited with 10 mg. and 1 mg. of Old Tuberculin (OT) one and two months after inoculation was significantly greater in animals inoculated with fresh BCG than with vaccine stored at either 20° or 37.5°C., and the allergic response diminished directly with the vaccine's exposure to higher temperatures (table 3). This point is especially demonstrated in the allergic response elicited three months after inoculation when the animals were tested with 0.1 mg. of OT. A qualitative difference in allergic reactions was noted in the animals inoculated with fresh and 20°C. one-week-old vaccines, namely, a greater tendency to central necrosis

TABLE 3

TUBERCULIN REACTIONS

AVERAGE VOLUME (MM.³)* OF INDURATION FORTY-EIGHT HOURS AFTER INTRADERMAL INJECTION OF TUBERCULIN IN GUINEA PIGS INOCULATED WITH BCG STORED AT 20° AND 37.5°C.

(Twelve Animals in Each Group)

Months After Inoculation	Dose of OT	BCG Vaccine									Controls
		Fresh	1-Week-Old		2-Week-Old		3-Week-Old		4-Week-Old		
			20°C.	37.5°C.	20°C.	37.5°C.	20°C.	37.5°C.	20°C.	37.5°C.	
	mg.										
1	10.0	846	635†	296†	360†	243†	167†	149†	169†	108†	—
2	1.0	661	453†	389†	401†	219†	308†	174†	329†	211†	—
3	0.1	199	173	160	113	74†	61†	45†	45†	31†	—
(After challenge)											
3	0.01	618‡	639‡	596‡	818‡	1,189	1,283	1,509	1,281	1,205	1,608

* Expressed in cubic millimeters of induration, calculated as half the thickness of the skin folded between caliper prongs multiplied by the two surface diameters of the tuberculin-indurated area (2 by 10 by 10 = 200 mm.³).

† Significantly different from fresh vaccine groups at the $P \leq 0.01$ level.

‡ Significantly different from the control group at the $P \leq 0.01$ level.

and regional lymphonodular hyperplasia on the third day after tuberculin testings, than in the remaining animals. When the animals were tested with 0.01 mg. of OT three months after the challenge inoculation with H37Rv (of established virulence), the degree of tuberculin allergy was significantly smaller in the groups of animals immunized with fresh, one-week-old 20° and 37.5°C., and two-week-old 20°C. vaccines than in the remaining groups, whose reactions were almost as strong as those noted in the control group.

Challenge infection: Thirteen weeks after inoculation with BCG vaccine, all of the immunized as well as the uninoculated control animals were given 0.01 mg. semidry weight of a four-week-old Löwenstein culture of H37Rv in the right hind leg by the intramuscular route. This dose gave rise to 10,194 eugonic colonies on a series of Löwenstein cultures after eight weeks of incubation. At this time there was no significant difference in the body weight among any of the ten groups of animals.

Results After Challenge Inoculation

Three months after the challenge inoculation, the average weight of the control animals had declined more than 200 gm., while the body weight was steadily increasing in the animals immunized with fresh, 20° and 37.5°C. one-week-old vaccines. The average body weight of the remaining animals was approximately 100 gm. less than that of the fresh vaccine group. Inguinal lymph nodes draining the virulent inoculum site in the control group were markedly enlarged and matted together, and almost half of the animals presented fistulated adjacent abscesses discharging caseum rich in acid-fast organisms. The virulent inoculum site in the immunized animals presented significantly smaller lymphonodular

hyperplasia in the groups inoculated with fresh, 20°C. one- and two-week-old, and 37.5°C. one-week-old vaccines than the remaining immunized animals, and fistulated abscesses were the exception rather than the rule in any of the immunized groups.

Seven control animals (58.3 per cent) died with generalized tuberculosis 97, 99, 114, 123, 129, 138, and 148 days after the challenge inoculation. The surviving five control animals were killed 148 days after the challenge inoculation, when the experiment was terminated. During this period no animal had died in the fresh, 20° and 37.5°C. one-week-old, and 20°C. two-week-old vaccine groups. One animal had died with generalized tuberculosis in each group immunized with the 37.5°C. two-week-old (114 days) and the 20°C. three-week-old (131 days) vaccines. Two animals died in each of the groups which received the 37.5°C. three-week-old (96 and 114 days) and the 20°C. four-week-old (108 and 114 days) vaccines. Five animals died in the group which received the 37.5°C. four-week-old (98, 116, 118, 124, and 131 days) vaccine. The surviving immunized animals were killed 150 to 162 days after the challenge inoculation.

Necropsy findings: Necropsy was delayed several hours until blood had coagulated to ensure uniformity in assessment of weight of lymph nodes and organs. The completed data were tested for significant differences by the method of Fisher and Yates (6) for comparison of two means. The body weight at necropsy was significantly greater in eight immunized groups than in the control animals, and the average weight was highest in the fresh vaccine group. The gross pathologic picture of localized and generalized tuberculosis suggests that the protection against virulent infection was greatest in the fresh vaccine group. In this group, 41.7 per cent presented no gross lesions, 33.3 per cent had only localized lesions, and 25 per cent had generalized tuberculosis. Effective resistance was apparently present in the 20° and 37.5°C. one-week-old vaccine groups, and slightly less in the 20°C. two-week-old vaccine group. The remaining immunized groups presented a gradually diminishing protection against the challenge inoculation.

The quantitative data on enlarged regional and distal (17 sets in each animal) lymph nodes and on the spleen, liver, and lungs agree closely with the gross pathologic picture. Arranged in order of significant tissue resistance against tuberculous hyperplasia assessed by actual weight of lymph nodes and organs, it would appear that the immunizing potency was highest in the group inoculated with fresh BCG vaccine, that it was slightly lower with 20°C. one- and two-week-old vaccines, and still lower with 37.5°C. one-week-old vaccine. It cannot be denied, however, that BCG vaccine stored at 20° and 37.5°C. for longer periods than two weeks is surely capable of producing specific resistance against a challenge infection. But, in comparison with the protective value of fresh vaccine and vaccine maintained for one week at 20°C., there would seem little justification for using vaccines consisting mostly of dead organisms.

DISCUSSION

Although BCG vaccine has been used internationally for more than two decades for protection against tuberculosis in man, it has not as yet attained the status of a standardized biologic product which invariably produces satisfactory

vaccination lesions and tuberculin hypersensitivity. Numerous uncontrollable factors affect the vaccine's antigenic potency from the time it is freshly prepared until it is implanted in man. Admittedly, the most damaging effects are produced by excessive temperature, light, and time of storage on the living population of BCG aggregates in the vaccine. It is important, therefore, to devise means to prevent such deterioration, since it is evident that the vaccine's optimal antigenic potency principally resides in the living bacillary aggregates.

When Calmette and Guérin distributed BCG vaccine in 1924 for general use, the vaccine was prepared with 25- to 35-day-old Sauton cultures in order to obtain maximum yield, and its expiration date was roughly fixed at two to three weeks. This vaccine was mostly administered by mouth, and subsequent tuberculin testing was not found necessary. Hence, it took some years before it was learned that such old Sauton cultures mainly consisted of dead BCG and that better parenteral vaccination lesions and subsequent tuberculin hypersensitivity could be obtained with vaccine produced with seven- to eleven-day-old Sauton cultures, which mainly consisted of living BCG aggregates. Furthermore, it became international usage to fix the expiration date of BCG vaccine at between seven and ten days after its manufacture when it contained an optimum of living elements. Aronson (8) set the expiration date to four to five days, at which time he found a significant decrease in the number of viable BCG.

In a group of 3,270 Danish schoolchildren vaccinated with BCG, Edwards and Gelting (2, 3) made use of vaccine stored up to seventy-five days at temperatures varying between 2° and 37°C. They observed no apparent reduction in the allergy-producing capacity of vaccine stored for at least two-and-one-half months at 2° to 4°C.; at room temperature (20°C.) for one month; at 30°C. for one week; and at 37°C. for five days. Unfortunately, their observation period of local vaccination lesions, adjacent lymphonodular enlargement, and tuberculin reactions with 5 and 10 tuberculin units (0.0001 and 0.0002 mg. of PPD) covers but ten post-vaccination weeks. A disturbing factor in their study is the disparity in time for reading tuberculin reactions, since some were read after three days and others after four days. Hence, we are informed that the stronger tuberculin reactions were produced by sixteen- to seventeen-day-old vaccine and the weaker with five- to six-day-old vaccine. In view of Aronson's (8) and the present writer's experience, this appears paradoxical since the former vaccine contains fewer living BCG elements than the latter. No mention is made of viability counts. This study thus furnished results which are contrary to general opinion and the writer's own experience. Accordingly, it seemed appropriate to undertake the present experimental series of studies of the antigenic potency of BCG stored at similar temperature variations, but also supplemented with vaccine consisting of killed or a mixture of living and killed BCG (1).

At the risk of being presumptuous, it is restated that the relation of allergy to immunity is not a simple quantitative function and that the one is not the measure of the other. The classical studies of Rich and colleagues (9), which the present writer was able to confirm (10), prove that immunity can be dissociated from allergy, and immunity may even function more effectively in the absence of allergy. Unfortunately, there is lacking a better gauge than the tuberculin

reaction to assess a satisfactory vaccination "take." In the experimental animal, however, the present study demonstrates that the degree of tuberculin hypersensitivity in fact foreshadows the degree of specific resistance elicited in protection tests. Correlation of these two factors alone, as produced by vaccines exposed up to four weeks at temperatures ranging from 2° to 37.5°C., under controlled experimental conditions, allows but one conclusion, namely, that the optimal antigenic potency of BCG vaccine is best preserved at 2° to 4°C. It cannot be denied that some degree of tuberculin hypersensitivity and resistance against a challenge infection may be produced by vaccines stored for several weeks at 20° to 37.5°C. But, inasmuch as the protection produced by vaccine stored up to two weeks at 2° to 4°C. produces only a partial resistance against a virulent challenge infection, this would seem the vaccine of choice, provided it is available.

SUMMARY

Freshly prepared BCG vaccine of standard strength loses half its viable elements after one week's storage at 20°C. in the dark, and nearly all after three weeks. Vaccine stored at 37.5°C. in the dark loses nearly all of its living population after one week.

Correlation is established between the numerical living aggregates of BCG in the vaccine stored at 20° and 37.5°C. and diminishing production of vaccination skin lesions, tuberculin hypersensitivity, and specific resistance against a challenge tuberculous infection in the guinea pig.

Storage of BCG vaccine at 2° to 4°C. remains the ideal condition for preserving its viable aggregates and optimal antigenic potency for at least two weeks after its preparation.

SUMARIO

El Efecto del Tiempo y de la Temperatura sobre la Potencia Antigénica de la Vacuna BCG: II. Conservación a 20° y 37.5°C.

La vacuna BCG recién preparada de potencia corriente pierde la mitad de sus elementos viables al cabo de una semana de conservación a 20°C. a obscuras, y casi todos al cabo de tres semanas. La vacuna conservada a 37.5°C. a obscuras pierde casi toda su población viva al cabo de una semana.

Existe correlación entre el número de elementos vivos de BCG en la vacuna conservada a 20° y 37.5°C. y la menor producción de lesiones cutáneas de vacunación, sensibilidad tuberculínica y resistencia específica a una infección tuberculosa provocadora en el cobayo.

La conservación de la vacuna BCG a 2° a 4°C. continúa siendo lo ideal para retener sus componentes viables y la potencia antigénica óptima a lo menos por dos semanas después de prepararla.

RESUME

L'action du temps et de la température sur le pouvoir antigène du vaccin BCG: II. Conservation à 20° et 37,5°C.

Le vaccin BCG fraîchement préparé et d'activité standard perd la moitié de ses éléments viables au bout d'une semaine de conservation à 20°C dans l'obscurité, et à peu près leur totalité au bout de trois semaines. Le vaccin conservé à 37,5°C dans l'obscurité perd la presque totalité de sa population viable au bout d'une semaine.

L'auteur établit une corrélation entre le nombre d'aggrégats vivants de BCG dans le vaccin conservé à 20° et à 37,5°C, la réduction des manifestations des réactions cutanées de la vaccination, la sensibilité à la tuberculine et la résistance spécifique contre l'infection tuberculeuse expérimentale chez le cobaye.

Le stockage du vaccin BCG à une température de 2 à 4°C demeure la condition idéale pour la préservation de ses aggrégats viables et de son pouvoir antigène optima pendant au moins deux semaines après sa préparation.

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Case Report

TUBERCULOUS PNEUMONIA DUE TO ORGANISMS RESISTANT TO STREPTOMYCIN AND ISONIAZID[1]

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In any case of tuberculous infection due to streptomycin-resistant organisms, it is conceivable that the tubercle bacilli might have been of the "wild type"[2] or they might have been transmitted by a patient whose strain of bacilli was predominantly resistant as a result of streptomycin therapy. It is recognized that, in "wild-type" populations of tubercle bacilli predominantly susceptible to streptomycin, a few cells may be found which are resistant to the drug. In general, however, these resistant cells do not become prominent in drug-susceptibility studies until their number increases significantly.

Cummings (1) summarized the results of a "screening test" for susceptibility to streptomycin performed on the strains of tubercle bacilli isolated from every patient admitted to thirty Veterans Administration hospitals. Among 1,405 patient-strains from persons who had had no previous streptomycin therapy, 3.6 per cent were streptomycin-resistant *in vitro*. To explain this relatively high incidence, it was speculated that some of these strains might represent examples of infection by streptomycin-resistant tubercle bacilli of the "wild type." An epidemiologic study was not done, however, to trace the contacts of these patients to ensure that they had not been exposed to tubercle bacilli from streptomycin-treated patients. A third possible explanation offered for this high value was that it fell within the range of laboratory error, considering that the tests were performed in thirty different laboratories. In a follow-up report presented in February, 1954, to the Thirteenth Conference on the Chemotherapy of Tuberculosis, Cummings stated that reinvestigation indicated that nine of the patients were found to have received streptomycin who were reported originally not to have received this medication. In addition, three had been incorrectly labeled in the laboratory. The final corrected value of 2.6 per cent was given to represent the incidence of patients with streptomycin-resistant organisms admitted to the thirty Veterans Administration hospitals. Arany (2) reported a case of tuberculous infection with streptomycin-resistant organisms and reviewed the twelve cases previously reported. It is striking that nine of the thirteen infections had occurred in hospital employees exposed to patients harboring streptomycin-resistant organisms. This writer also mentioned that there was no incidence of strains initially resistant to streptomycin in more than 400 other cases of tuberculosis studied. Furtos and Doane (3) did not observe any instance of initially high bacterial resistance to streptomycin in 385 patients. This corresponds to the experience at the District of Columbia General Hospital, where drug-susceptibility studies are performed routinely.

The possibility of the transmission of streptomycin-resistant tubercle bacilli points up

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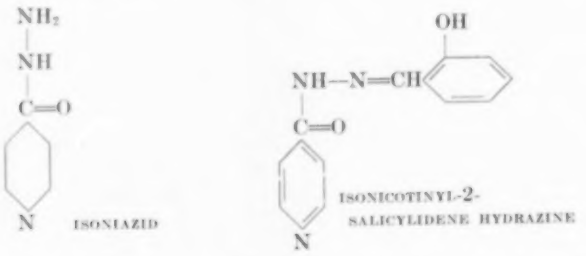
[2] The term "wild-type" tubercle bacilli designates the bacilli as they have occurred in nature, i.e., without exposure to antimicrobial drugs.

a potential public health hazard. The recalcitrant, who repeatedly leaves the hospital against medical advice, may be responsible for the dissemination of streptomycin-resistant tuberculosis.

The present writers have been impressed by the occasional isolation of tubercle bacilli resistant to 1 γ or more of isoniazid per ml. from patients who have never received this drug. Hobby and Lenert (4) have reported that tubercle bacilli resistant to isoniazid existed in significant numbers in strains of tubercle bacilli of human origin which had had no previous contact with this drug.

The presence of isoniazid-resistant bacilli in "wild-type" strains of *M. tuberculosis* may adversely affect the usefulness of this drug as an antituberculous agent. Similarly, the emergence of isoniazid-resistant organisms during treatment with this drug has been reported to be associated with a failure in roentgenographic or bacteriologic improvement even though general clinical improvement may be maintained (5).

The following case of tuberculous pneumonia is interesting because tubercle bacilli resistant to both streptomycin and isoniazid were isolated from a patient who had never received either drug. Furthermore, a careful epidemiologic study failed to disclose any contacts who had received these drugs and who might subsequently have transmitted the resistant organisms to the patient. The isoniazid salicylate compound used in the patient is isonicotinyl-2-salicylidene hydrazine.[3] It has the following structural formula, which is compared with isoniazid:



ISONIAZID

ISONICOTINYL-2-SALICYLIDENE HYDRAZINE

The present writers have reported (6) that this agent is as useful as isoniazid, is less toxic, and at times is effective even in the presence of infection due to tubercle bacilli which have become resistant during isoniazid therapy. However, cross-resistance in which tubercle bacilli resistant to isoniazid are likewise resistant to the other compound has been common in the writers' experience.

CASE REPORT

Ten days prior to admission, a 31-year-old Negro female noted the onset of a "head cold" associated with malaise and headache and followed by fever and a productive cough. Two days later the cough became productive of yellow sputum, and she visited the outpatient clinic of another hospital. She was considered to have pneumonia and was given repository penicillin and sulfadiazine. Her symptoms became worse, and hospitalization was recommended. At the time of admission to the District of Columbia General Hospital, her past history did not reveal evidence of chronic bronchopulmonary disease. Before the onset of her present illness, there was nothing to suggest chronic illness, such as malaise, fatigue,

[3] Supplied as Salizid, previously known as Compound 1595, by Dr. George Mast, Nepera Chemical Co., Yonkers, New York.

weakness, or weight loss. A searching epidemiologic study did not uncover any source of tuberculous infection.

Although she was well developed and well nourished, the patient looked seriously ill. She had moderate respiratory distress and had an annoying cough. There was no palpable lymphadenopathy. The ocular fundi were normal. Slight pharyngeal injection and enlargement of the tonsils were noted.

The chest was symmetrical. There was decreased respiratory excursion on the right side, with signs of consolidation of the right lower lobe. Examination of the heart revealed no abnormalities. The liver was slightly enlarged. The blood pressure was 108 systolic, 76 diastolic in mm. of mercury; the pulse rate was 120 per minute; the respiratory rate, 28; and the temperature, 103.2°F.

The leukocyte count was 9,400 per cu. mm., with 79 per cent neutrophils, 15 per cent lymphocytes, 4 per cent monocytes, and 2 per cent eosinophils. Hemoglobin was 12 gm., and the hematocrit was 38 mm. The corrected erythrocyte sedimentation rate was 18 mm. per hour (Wintrobe method). The icterus index was 2, and urinalysis was negative.

Microscopy of the sputum revealed gram-positive cocci in pairs, chains of gram-positive bacilli, and gram-negative diplococci. Culture of the sputum showed hemolytic streptococci and *Neisseria catarrhalis*. Multiple smears of sputum examined for tubercle bacilli were negative. Blood cultures were sterile.

A roentgenogram of the chest (figure 1) showed a homogeneous density involving the lower half of the right lung and a mottled density of the upper half of the right lung and the lower three-fourths of the left lung. There was also suggestive evidence of fluid in the right pleural cavity.

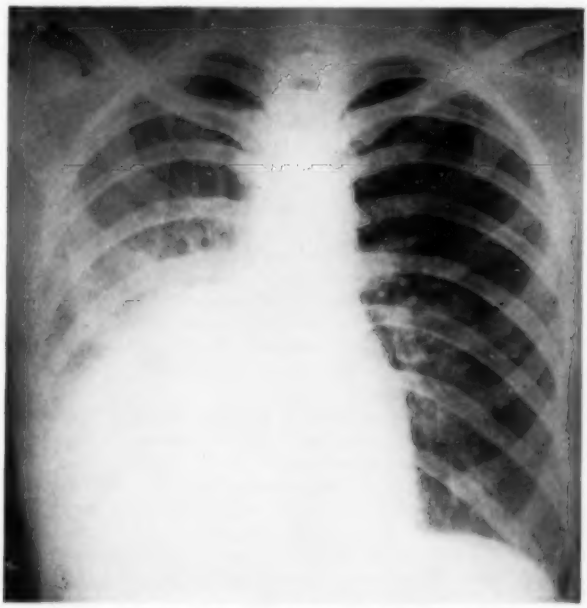


FIG. 1. Roentgenogram of the chest taken on admission showing consolidation of the lower half of the right lung and mottling of the upper half of the right lung and lower three-fourths of the left lung. There is also a suggestion of fluid in the right pleural space.

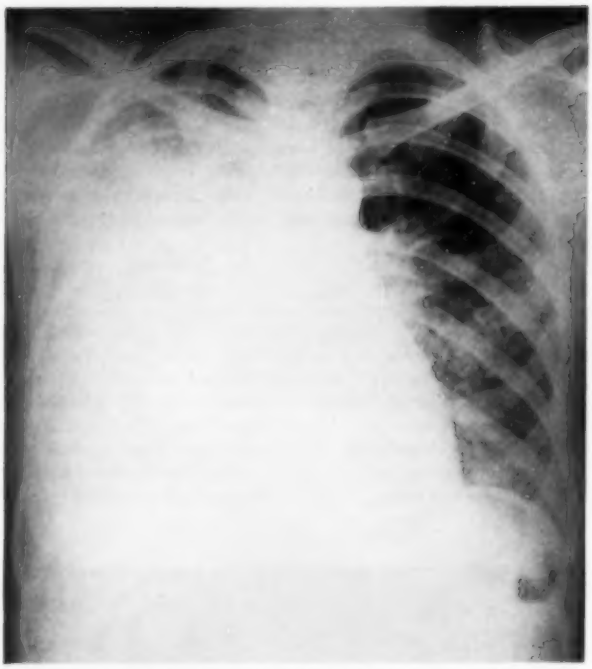


FIG. 2. Roentgenogram of the chest taken eleven days later showing massive consolidation of the right lung, right pleural effusion, and an increase in the mottling in the left lung.

The initial treatment was based on the clinical diagnosis of pneumonia. The patient was given carbomycin, 500 mg. four times daily for two days. This was changed to penicillin, 500,000 units of a delayed action preparation twice daily, to which a sulfonamide, 1.0 gm. three times daily, was added four days later. Eight days after admission, penicillin and the sulfonamide were stopped because of failure of response and because of roentgenographic evidence of progression of the pneumonia, and oxytetracycline (Terramycin®), 500 mg. every four hours, was begun. Three days later this dose was increased to 750 mg. every four hours. For the first twelve days of her hospitalization, therefore, she was treated as though she had nontuberculous pneumonia.

During this time the patient continued to be critically ill, with temperature ranging up to 105°F., persistent dyspnea, and clinical and roentgenographic evidence of advancement of the disease process, with involvement of the entire right lung and an increase in the lesion in the left lung. In spite of these objective changes, the patient persistently claimed to be feeling better—a euphoric subjective state often noted in patients with extensive tuberculosis. Her leukocyte count remained below 10,000 per cu. mm., with 80 per cent neutrophils. Repeated sputum cultures revealed no significant information, and repeated blood cultures were sterile. There were no cold agglutinins, and the agglutination tests usually made in cases of unexplained fever were negative. Ten concentrated smears of sputum during this time were negative for tubercle bacilli. Her skin test with intermediate-strength purified protein derivative (PPD) was negative.

Eleven days after admission, on November 14, 1952, the patient was seen in consultation by members of the Pulmonary Disease Division. Although the diagnosis of pulmonary

tuberculosis had not been established bacteriologically, it was thought that treatment for tuberculosis should be started because of her critical state. Accordingly, she was given 1.0 gm. of streptomycin every third day and 100 mg. of the isoniazid-salicylate compound every six hours. This regimen had been extremely successful in other patients with acute tuberculous pneumonia (6). Oxytetracycline, 4 gm. daily, was continued, with the addition of aerosolized bronchodilators and aerosol oxytetracycline. A roentgenogram of the chest at this time (figure 2) showed an extension of the disease bilaterally.

There was no evidence of improvement during the next five days. On November 19, 1952, a right thoracentesis was performed and 75 cc. of serosanguineous fluid were removed. The right lung was aspirated in an attempt to recover an organism. Smears of this material and cultures were negative for tubercle bacilli. As a result of the procedure the patient developed approximately a 10 per cent pneumothorax, which did not influence her clinical state. The following day she was bronchoscoped. The mucosa of the tracheobronchial tree was diffusely inflamed, and purulent secretions were obtained from both lower lobe bronchi, but there was no evidence of bronchial obstruction.

On November 21, 1952, in spite of persistently negative smears for tubercle bacilli and her negative PPD reaction, oxytetracycline was stopped because of the conviction that a fulminating tuberculous pneumonia was present. Specimens of sputum submitted on November 20 and 21, 1952, were finally found positive for acid-fast bacilli on microscopy. The result was reported on November 24. The patient's condition deteriorated further. A chest roentgenogram showed further advancement, with bilateral cavitation (figure 3). She died on December 3, 1952.

Post-mortem examination: At autopsy, the significant findings were limited to the thorax.

FIG. 3. Roentgenogram of the chest taken one month after admission, just prior to death, showing extensive bilateral involvement and cavitation.

TABLE 1

DRUG-SUSCEPTIBILITY STUDIES ON TUBERCLE BACILLI CULTURED FROM SPUTUM AND BONE MARROW DURING LIFE

	Streptomycin γ per ml.		Isoniazid γ per ml.		Isoniazid Salicylate γ per ml.		PAS mg. per 100 ml.		Nicotinaldehyde Thiosemicarbazone γ per ml.		Streptomycin and Isoniazid		Streptomycin and Isoniazid-Salicylate Compound	
	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible
Sputum, 11-4-52	30	100	50	100	5	20						0.3		0.3
												0.06		0.06
Sputum, 11-5-52	30	100	20	50	5	20	0.1	1		1		0.3		0.3
												0.06		0.06
Sputum, 11-10-52	30	100	20	50	5	20	0.1	1		1		0.3		0.3
												0.06		0.06
Sputum, 11-14-52	30	100	20	50	5	20	0.1	1		1		0.3		0.3
												0.06		0.06
Sputum, 11-18-52	30	100	20	50	5	20	0.1	1		1		0.3		0.3
												0.06		0.06
Bone marrow, 11-18-52	100	300	50	100	50	100	0.1	1		1		0.3		0.3
												0.06		0.06

TABLE 2

DRUG-SUSCEPTIBILITY STUDIES ON BACILLI ISOLATED FROM AUTOPSY MATERIAL

	Streptomycin γ per ml.		Isoniazid γ per ml.		Isoniazid Salicylate γ per ml.		PAS, mg. per 100 ml.		Nicotinaldehyde Thiosemicarbazone γ per ml.		Streptomycin and Isoniazid	
	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible
Right main stem bronchus	100	300	100	150	100	150	1	10	1	5	10	30
											0.3	1
Left main stem bronchus	100	300	100	150	100	150	1	10	1	5	10	30
											0.3	1
Right lung cavity	30	100	100	200	50	100	0.1	1	1	5		0.3
												0.06
Left lung cavity	100	300	100	200	50	100	0.1	1	1	5		0.3
												0.06
Right lung pneumonic lesion	30	100	50	100	50	100	0.1	1	1	5	0.3	10
											0.06	0.3
Left lung pneumonic lesion	100	300	100	200	50	100	0.1	1	1	5		0.3
												0.06

There were 500 cc. of bloody fluid in the right pleural space. The larynx and trachea were essentially normal. The mediastinal lymph nodes were greatly enlarged.

The right lung was voluminous and did not collapse. It weighed 1,070 gm. The pleural surface was cloudy and showed scattered dark red hemorrhagic areas measuring up to 6 cm. in diameter. The entire lung was solid. On section, there were numerous small, irregular, firm, grayish areas, 0.5 cm. to 1.5 cm. in diameter, scattered throughout all lobes. Many of

these showed central caseation with cavity formation. The surrounding parenchyma consisted of a narrow margin of beefy red tissue. The bronchial mucosa was covered by a thick, greenish, odorless material. The mucosa itself was markedly congested. The major pulmonary vessels and their branches were patent throughout.

The left lung weighed 900 gm. and was essentially similar to the right in its gross appearance.

Microscopic examination showed a diffuse lobar pneumonia in which the alveoli were filled with a pink amorphous material containing numerous monocytes and moderate numbers of lymphocytes and eosinophils. Early fibroblastic proliferation was also found in the alveolar exudate. Focal areas of early caseation necrosis were present, as well as abscesses in which the exudate consisted principally of polymorphonuclear cells with an acidophilic cytoplasm. Around the areas of frank caseation, early epithelioid organization such as is seen in early tubercle formation was observed. An additional finding in the lungs was that many of the abscess cavities contained large colonies of mycelia which were histologically identified as *Candida albicans*. Culture of these areas also revealed *Candida albicans*. Acid-fast stains of the tissues revealed numerous acid-fast bacilli.

The final pathologic diagnosis was (*1*) bilateral acute tuberculous pneumonia, (*2*) right hemothorax, (*3*) reactive lymphadenopathy, generalized, (*4*) visceral congestion and edema, (*5*) acute miliary tuberculosis.

Special Bacteriologic Studies

A summary of the drug-susceptibility studies performed on the organisms isolated from sputum and bone marrow during life but reported after death is shown in table 1. All specimens showed tubercle bacilli which were resistant to 30 γ or more of streptomycin per ml., 20 γ or more of isoniazid per ml., and 5 γ or more of the isoniazid-salicylate compound per ml. The organisms were completely inhibited by 0.3 γ of streptomycin per ml. and 0.06 γ of isoniazid per ml. when both agents were incorporated in the culture medium. This was also true when both streptomycin and the isoniazid-salicylate compound were used in the culture medium. It is interesting to note that the tubercle bacilli isolated were susceptible to nicotinaldehyde thiosemicarbazone but somewhat resistant to para-aminosalicylic acid (PAS).

In table 2 the drug-susceptibility data on the bacilli cultured at autopsy are summarized. The marked resistance to streptomycin, isoniazid, and the isoniazid-salicylate compound of the tubercle bacilli isolated from all areas of the lung is apparent. It is striking to note that, in spite of the marked resistance to these drugs when used alone, the tubercle bacilli were inhibited by subinhibitory concentrations of streptomycin and isoniazid when these agents were used in combination in the culture medium. This was also true when both streptomycin and the isoniazid-salicylate compound were used in the culture medium.

All the microorganisms isolated showed the morphologic and colonial characteristics of *M. tuberculosis*.

Special Virulence Studies

In order to determine whether or not the isoniazid-resistant tubercle bacilli isolated from the patient were virulent for guinea pigs, special studies were done.

Four guinea pigs which were tuberculin-negative were infected by the subcutaneous injection of 0.1 ml. of a six-day-old culture of the patient's strain of tubercle bacilli grown in Dubos liquid medium. These tubercle bacilli were resistant to 20 γ of isoniazid per ml., to 30 γ of streptomycin per ml., and to 5 γ of the isoniazid-salicylate compound per ml. On the ninth day following the injection, all of the animals were febrile. Striking weight loss occurred during the period of observation. On the twenty-ninth day, one

animal died. At autopsy, extensive (4-plus) disease was found in the liver, spleen, lungs, and lymph nodes. The remaining 3 guinea pigs were sacrificed on the thirty-first day. All were found to have extensive (4-plus) disease in the spleen, liver, and lymph nodes. One had extensive (4-plus) disease in the lungs, while in 2 animals there was moderately extensive (2-plus) disease in the lungs. Cultures of each of the organs yielded tubercle bacilli having the same range of drug-susceptibility as was present in the injected tubercle bacilli.

Three groups of 4 guinea pigs each were inoculated with organisms obtained at autopsy from three different portions of the lungs of the patient. These 12 animals were sacrificed on the thirty-first day and were found to have extensive (4-plus) disease in the liver, spleen, and lymph nodes and a varied amount of disease (1-plus to 4-plus) in the lungs. Cultures were made at autopsy from each of the organs and yielded tubercle bacilli with the same degree of drug-resistance present in the originally injected organisms.

The possibility existed that the tubercle bacilli present in the sputum and in the lung represented a mixed population of isoniazid-susceptible and isoniazid-resistant organisms as well as some bacilli susceptible to, and some resistant to, the isoniazid-salicylate compound. If this were true, the tuberculosis in the infected guinea pigs could have been produced by the drug-susceptible members of the populations. For this reason, 9 guinea pigs which were infected with tubercle bacilli resistant to 50 γ of isoniazid per ml., to 30 γ of streptomycin per ml., and to 5 γ of isoniazid-salicylate compound per ml. were treated with the isoniazid-salicylate. The same severe tuberculosis developed in these guinea pigs as did in the untreated controls. This would seem to indicate that the same tubercle bacilli which were resistant to the isoniazid-salicylate compound were pathogenic for guinea pigs.

A series of guinea pigs similarly infected is currently being treated with isoniazid to determine with certainty the virulence of the isoniazid-resistant tubercle bacilli. It is anticipated that the results will be similar to those observed with the isoniazid-salicylate therapy.

DISCUSSION

There is evidence that pathogenic tubercle bacilli which exhibit increased resistance to streptomycin *in vitro* will not be influenced by the use of this drug *in vivo*. Feldman and his associates (7) demonstrated that streptomycin was not therapeutically effective in the guinea pig if the tuberculosis was induced by a highly resistant strain of tubercle bacilli. Youmans and Williston (8) showed that mice which were infected with streptomycin-resistant tubercle bacilli did not respond to treatment with streptomycin. Steenken and Wolinsky (9) likewise demonstrated that guinea pigs infected with two highly streptomycin-resistant strains of tubercle bacilli derived from humans were not benefited by streptomycin therapy. Their studies also revealed that, when guinea pigs were infected with artificial mixtures containing drug-susceptible and -resistant tubercle bacilli in varying proportions, the animals showed improved response to treatment as the percentage of resistant organisms decreased below 10 per cent. There is also good evidence (10, 11) that tuberculous patients who develop organisms which are streptomycin-resistant *in vitro* may cease to respond favorably to streptomycin or may show exacerbations of disease. Furthermore, re-treatment with streptomycin in the presence of a predominantly drug-resistant bacterial population has been unsatisfactory (10, 11).

Infection due to streptomycin-resistant organisms may respond to treatment with para-aminosalicylic acid (PAS) alone. More commonly, however, this type of infection

is more effectively treated with both streptomycin and PAS. When the infecting organisms are resistant to both streptomycin and PAS, regular response to the use of these drugs is not to be expected.

Isoniazid is valuable in the therapy of tuberculosis due to isoniazid-susceptible organisms, streptomycin-resistant, or PAS-resistant organisms. There is evidence that streptomycin along with isoniazid is more valuable in the therapy of streptomycin-susceptible, streptomycin-resistant, isoniazid-resistant, or isoniazid-susceptible tuberculosis than either streptomycin or isoniazid alone. With this combination therapy, there is a higher rate of sputum "conversion" and a lower incidence in the appearance of organisms resistant to streptomycin and/or isoniazid (12, 13).

The twice-weekly administration of streptomycin combined with daily isoniazid or the isoniazid-salicylate compound is effective in the management of acute tuberculous pneumonia (13, 6). In these patients the tubercle bacilli are usually susceptible to these drugs, at least initially. It was not surprising in the case reported that therapy was ineffective in the presence of organisms resistant to both streptomycin and the isoniazid-salicylate compound, although, to be sure, the susceptibility data were not available until after the patient's death. Had there been knowledge of resistance of the bacilli to streptomycin and to the isoniazid-salicylate compound, other combinations of therapeutic agents would have been used.

A loss of virulence for guinea pigs has been demonstrated for isoniazid-resistant tubercle bacilli (6, 14). However, not all isoniazid-resistant tubercle bacilli have lost their virulence, for several studies (15, 16, 17) describe virulent tuberculosis in guinea pigs and mice infected with isoniazid-resistant organisms. Furthermore, exacerbations of tuberculosis have been demonstrated in patients whose isoniazid-resistant organisms showed very slight or no virulence for guinea pigs (14). Certainly, there can be little question of the virulence of the isoniazid-streptomycin–resistant organisms in the present case.

In spite of the failure to discover a tuberculous contact in this case, it seems unlikely that the tubercle bacilli were natively resistant to both streptomycin and isoniazid. Because the initially isolated tubercle bacilli were also somewhat resistant to PAS, it would appear more logical to ascribe the bacillary resistance to transmission of tubercle bacilli by a patient whose organisms were made resistant by exposure to the antituberculous agents. It is not uncommon to see patients who have received streptomycin and PAS by an unacceptable regimen who have had a relapse under therapy concomitant with the appearance of drug-resistant strains of tubercle bacilli. With the advent of isoniazid, this drug alone was not infrequently given to these patients. The appearance of isoniazid-resistant tubercle bacilli is regularly anticipated when isoniazid is used alone. As a matter of fact, when patients receive streptomycin and isoniazid concurrently, strains resistant to both drugs will eventually develop provided the bacillary discharge continues (18). Although purely speculative, it is suggested that the patient who is the subject of this report had an unrecognized contact with a tuberculous patient who had received streptomycin, PAS, and isoniazid to the point of the development of strains of tubercle bacilli resistant to these drugs.

As *M. tuberculosis avian* is resistant to most antituberculous drugs and because progressive tuberculosis in man due to this organism has been reported (19, 20), fowl have been injected to determine this possibility. However, the marked pathogenicity of the isolated organisms for guinea pigs makes the likelihood of infection due to avian-type tubercle bacilli rather remote.

SUMMARY

A fulminating case of tuberculous pneumonia is reported in which the initially isolated tubercle bacilli were resistant to streptomycin, isoniazid, and PAS. The tubercle bacilli, although isoniazid-resistant, were markedly virulent for guinea pigs.

It appears likely that the bacillary resistance was due to transmission of tubercle bacilli by a patient whose strain of tubercle bacilli was drug-resistant as a result of treatment with streptomycin, isoniazid, and PAS.

SUMARIO

Neumonía Tuberculosa Debida a Microbios Resistentes a la Estreptomicina y la Isoniacida

Preséntase un caso fulminante de neumonía tuberculosa en el que los bacilos tuberculosos aislados inicialmente eran resistentes a la estreptomicina, la isoniacida y al PAS. Aunque isoniacidorresistentes, los bacilos eran notablemente virulentos para los cobayos.

Parece que la resistencia bacilar se debía a transmisión de bacilos tuberculosos por un enfermo cuyos microbios habían formado resistencia a consecuencia del tratamiento con estreptomicina, isoniacida y PAS.

RESUME

Pneumonie tuberculeuse due à des germes résistant à la streptomycine et à l'isoniazide

Un cas de pneumonie tuberculeuse foudroyante est rapporté. Les bacilles tuberculeux initialement isolés étaient résistants à la streptomycine, à l'isoniazide et au PAS. Ces bacilles tuberculeux résistants à l'isoniazide étaient cependant extrêmement virulents pour les cobayes.

Il semble que la résistance bacillaire soit imputable à la transmission des B.K. par un malade dont les bacilles ont acquis une résistance à la suite du traitement par la streptomycine, l'isoniazide et le PAS.

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Editorial

The Problem of Irregular Discharge

Readers of this journal need no introduction to the vast medical-social problem created by irregular discharge of tuberculous patients. From the literature available, it is evident that past work in this field generally has suffered from a lack of systematic investigation of large numbers of discharges, of serial admissions, and the absence of integrated team work by various disciplines. The results of previous studies usually have reflected the bias of one or another professional group. Furthermore, the recommendations or conclusions derived from these works too often have been unproved, unrealistic, or merely "seat of the pants" solutions.

Thus, there still are a number of pressing needs in relation to the problem of irregular discharge. The first is for an accurate identification of the nature of the problem, and, if possible, its cause. The second is for some means of detection and prediction of those patients who are likely to resort to irregular discharge. The third is for practical measures, which are generally applicable, for carrying out this process of early detection and for "retrieving" patients who threaten or attempt to leave the hospital before discharged with medical sanction.

Early in 1952, a research group at the Madison Veterans Administration Hospital began preliminary surveys along the aforementioned lines. From the first, the investigation was carried out by a small team (internists, social workers, psychiatrists, and pyschologists) cooperating actively with a larger group consisting of members of all departments of the hospital. This research project has had several main limbs. It has centered about careful review of all irregular discharges, detailed study of patients who threaten irregular discharge, similar study of comparison groups of patients who remained to receive regular discharge, and continuous participation by the staff in attempts at solutions to the problems turned up in the process of the investigation. This method, called "action research" in some quarters[1], also has involved close scrutiny of staff attitudes and actions in so far as they affect irregular discharges.

Early discussion by the group revealed a multitude of "answers" to the reasons for, and solutions to, the problem of irregular discharge. Most of these "answers" can be heard in any tuberculosis hospital, but have never (so far as we know) been subjected to critical evaluation. It was decided, therefore, that in the beginning a "clearing way" period was necessary. Accordingly, weekly conferences were held wherein each case of irregular discharge was discussed in detail by all members of the hospital staff who had contact with the patient. In addition, patients who were serious risks from the standpoint of irregular discharge were discussed in like manner.

[1] Lippitt, R.: Field theory and experiment in social psychology: Autocratic and democratic group atmospheres, Am. J. Sociol., 1939, *45*, 26.

A second phase of this preliminary survey involved the comparison of the first 100 irregular discharge patients and the first 100 maximum hospital benefit discharge patients from this hospital with respect to age, race, marital status, service connection, distance of the hospital from the patient's home, activity of tuberculosis, extent of tuberculosis, pulmonary surgery, occupation, length of hospitalization, and history of previous irregular discharge. Recently, the results of this study have been reported.[2]

In summary, it has been shown that there are no statistically significant differences between these two groups except for the factors of pulmonary surgery, occupation, length of hospitalization, extent of disease, and history of previous irregular discharge. The latter three factors were shown to have a differential significance at the 0.01 level of confidence or beyond. They also permit tentative hypotheses in regard to estimating a patient's proneness to irregular discharge. It appears that patients who have resorted to irregular discharge before and those who have far advanced disease (or, especially, patients in whom *both* factors are present) are much more likely to leave the hospital irregularly than the average patient.

Some interesting data regarding length of hospitalization and irregular discharge also appeared. Forty per cent of the irregular discharges in the reported series occurred within the first three months of hospital stay. Length of hospitalization for the irregular discharge patients was considerably less than for the regularly discharged patients. Irregular discharge patients return as well as leave the hospital, and there is a definite (and roughly predictable) period within which preventive measures can be instituted before irregular discharge is likely to occur. Further analysis of statistical data promises sharpening of such predictions, as well as identification on admission of many potential "problem" patients.

As the harvest from the first cast of the investigative net into such factors as those noted above was found to be relatively meager, it was obvious that more penetrating observation was in order. Earlier studies uniformly have indicated situational and emotional stress (expressed in various terms) as an important cause of irregular discharge. Review in the irregular discharge conferences of case after case of irregular discharge tended to confirm this view, but a bewildering variety of forces began to appear. Thus, conflicts which arose on the ward between staff and patient or patient and patient came into focus, together with problems which patients had involving difficulty with their families and home communities. It became clear that there are many different types of irregular discharge (single or multiple, planned or impulsive) which differ greatly, depending on the stage of hospitalization during which they occur. The precipitating circumstances vary for each type. Furthermore, the response of individual patients to similar circumstances has tremendous variability. It became evident that patients who resorted to irregular discharge were contending with extra-

[2] Lorenz, T. H., Lewis, W. C., Calden, G., and Thurston, J. R.: Investigation of irregular discharge of tuberculosis patients: Report of a preliminary study (submitted for publication).

hospital problems, had intra-hospital conflicts, and (with few exceptions) could be categorized quite readily from a neuropsychiatric standpoint.

In an effort to assess the significance of these complex and interacting factors, a comparison group was sought and found in the patients who had been referred for evaluation to the Psychosomatic Section of the hospital because of situational and emotional problems, but who had remained hospitalized—in contrast to the irregular discharge patients. In general, no significant differences could be found between these two groups of disturbed patients in regard to the type or degree of problems they had outside of the hospital or the conflicts they were contending with inside of the hospital. Comparison of the gross neuropsychiatric diagnostic categories in the two groups was similarly unrewarding. The majority of patients in both series were diagnosed as having character disorders of various types; approximately one-third had psychoneuroses and a few were psychotic. (It is to be noted that certain reservations are in order with respect to the foregoing. As far as both stress factors and neuropsychiatric diagnoses are concerned, it is recognized that differences probably exist between irregular discharge patients and others who do not resort to irregular discharge. However, these differences have not been detectable at the level of the present study.)

As the investigation progressed, some general theoretical formulations began to appear which provided a rationale for understanding irregular discharge. It can be stated without hesitation that there is no one type of irregular discharge patient or "irregular discharge" situation. It seems to us that irregular discharge can best be understood against the general background of the usual course of hospitalization for any patient.[3]

There is a sort of "natural history" of hospitalization for tuberculosis. Briefly stated, patients are forced by present methods of therapy to undergo what psychiatrists would call an "artificial regression" (more properly an *iatrogenic* regression). Patients are first made into something approximating infants, cared for by the hospital "family" (with mother, father, grandfather, et cetera, substitutes), and only gradually are they allowed to creep, walk, and flex their adolescent muscles. Most patients are aware of this, clearly or dimly, and likewise are aware of special problems which arise at each stage of the "growing up" process.

Early in hospitalization, patients experience a sense of lonely isolation. The usual sources of self-esteem (work, responsible participation in family and community life, et cetera) are denied them. Emotional attachments to significant persons are disrupted. Anxiety is stimulated by the awesome prospects of their disease, of surgery, and even of life as an "arrested" patient. The usual devices for relieving anxiety are markedly minimized by enforced bed rest and other restrictions related to the treatment program. What is required is an adjustive effort on the part of the patient, which is successful surprisingly often in the "normal" person. New attachments are made to the hospital staff and to other patients, problems are "talked out" with various people, and constructive

[3] Lewis, W. C., Lorenz, T. H., and Calden, G.: A major unsolved problem: Irregular discharge from tuberculosis hospitals, Psychosom. Med. (to be published).

solutions for family difficulties are reached. Various devices such as occupational therapy, educational therapy, reading, et cetera, are used to control anxiety, excessive fantasy, and boredom. Any interference with this adjustment process at any stage of hospitalization may result in symptoms indicative of a disturbed emotional economy. We regard the desperate measure of irregular discharge as one such symptom.

Irregular discharges in the *early* period of hospitalization seem to center around several types of problems. Realization of their plight, i.e., tuberculosis produces shock, apathy, and depression in most patients. In keeping with this, there is increased need on the part of these patients for the support of loved ones. To some people, separation from loved ones is such a devastating blow to their security that it cannot be endured. A 60-year-old man who had never been away from his wife for a single night since their marriage told us he was leaving against advice a few days after admission. He said, "I just want to go home to mamma," and he could not be dissuaded. Such a reaction reminds one of the behavior seen in frightened children in situations involving separation from mother. Emotionally healthy patients usually find ways to make such separations tolerable (new friends, reliance upon staff, et cetera). On the other hand, the lack of inner security found among many irregular discharge patients, which makes separation from loved ones such a problem, also makes these patients less able to develop new attachments.

All irregular discharge patients do not react early in hospitalization in the fashion just described. An even more characteristic pattern found among our series of patients is that of "wandering in as strangers" and remaining so, until they wander out again. These irregular discharge patients under-react from an emotional standpoint. Many of them complete the item on the Madison Sentence Completion Form[4] which begins "What I'll miss the most when I leave the hospital," with the statement: "Nothing at all." Close study of these people reveals a pattern of unstable and difficult relationships in early childhood, which are repeated later in adolescence and adulthood and are reflected in indifferent attitudes toward the hospital staff. Characteristically, these patients respond to minor interruptions or interferences with their flimsy attachments to doctors and nurses by quietly "walking away" from the hospital. Vacation periods involving absences of staff members, shifts of personnel from ward to ward, changes in staff assignments, et cetera, are sufficient provocation for irregular discharge on the part of these "isolated" persons.

It would be relatively easy to deal with irregular discharge patients if one were confronted merely by the problems of loneliness and isolation. An additional complexity exists however: The phenomenon of irregular discharge, in a large number of cases, is more closely related to *conflicts* about attachments than to *lack* of attachments. Frequently, irregular discharge patients care enough about interpersonal relationships to become hurt and spiteful at any denial or frustration in this area. These patients over-react emotionally and they "run out" of the

[4] CALDEN, G.: A method for evaluating the attitudes of tuberculous patients, Am. Rev. Tubere., 1953, *67*, 722.

hospital impulsively. There are, of course, many "touchy spots" for patients of this type. Generally, these can be demonstrated to center around reactivated emotional conflicts of childhood. The person who has been relatively deprived of childhood dependency, or has had to renounce it, is apt to become anxious when he is encouraged to "relax" and be taken care of. Old rivalry situations in the family tend to recur in the hospital in respect to other patients. Men who always fought a stern father fight a stern doctor. Those who had an indulgent mother expect the same indulgence from the ward nurse.

At later periods in hospitalization, different problems arise which provoke irregular discharge. Whereas early tensions often center about loneliness, when patients get up and around (commensurate with medical improvement), former sensitive areas seem to disappear only to be replaced by others. Problems of adjustment during early stages of hospitalization seem to be related to infancy and childhood and to interferences with early basic relationships to people. Later problems during the ambulatory stage are more like those seen in adolescence. Scuffles, gangs, defiance, prestige problems, and competition appear more and more. The doctor becomes an obstruction to liberty and self-assertion. Multiple defiant irregular discharges are not infrequent in this period (six to twelve months). Toward the end of the treatment program, when patients are living by a nearly normal schedule of activity, irregular discharge seems to focus more about financial and job difficulties. In general, the later the irregular discharge, the more "mature" flavor it has.

Obviously, solutions must fit problems. We have found that the maladjustment which occurs during the early period of hospitalization tends to respond to efforts directed at diminishing emotional isolation. The greater liberality of small tuberculosis hospitals in permitting family visits and use of symbolic possessions (as important to the mature individual in promoting a feeling of being "at home" as a teddy bear is to the lonely child) and the greater stability of ward attachments in these hospitals seem significant to us in relation to their generally lower irregular discharge rates. Even in large institutions these measures can be employed in selected cases. Occasionally, bed-rest rules have been modified in order to diminish the emotional vacuum some patients experience on absolute bed rest. Although fraught with danger unless used with discretion, in a few cases completion of treatment (interrupted repeatedly in the past through irregular discharge) was made possible by this means. Transfers from ward to ward need to be minimized. Positive attachments to any staff member (chaplain, aide, nurse, social worker, and doctor) should be detected and fostered. The greater the understanding which can be developed in the hospital "family" for the areas of emotional conflict in patients, the better able the "family" is to deal with these problems. In this regard, the Madison Sentence Completion Form is of great help. It serves as a device for detecting high potential irregular discharge risks but, furthermore, it points up vulnerable spots in the makeup of patients which can be manipulated in favor of better hospital adjustment. Ward conferences, including all staff members in contact with patients, also are of great help in developing constructive plans for coping with irregular discharge problems and in sharpening the sensitivity of the staff to such problems.

For the later period of hospitalization, when "late childhood and adolescent" difficulties become prominent, patient self-government appears to be most promising. The greater the control of the patient group *by* the patient group, the greater is each patient's sense of mature responsibility toward himself and the hospital. Although group therapy has shown evidence of its usefulness, both in this hospital and in others, this cannot replace the value of active responsible participation of the patients in the rule-making and disciplinary aspects of their relations with the hospital. It is, after all, parallel with the family; one would not expect mature behavior from a young adult unless some responsibility were given gradually to him as he approached the time when he was to leave home. Direct efforts opposed to irregular discharges can be expected by members of a patient council when their cooperation is either allowed or sought. In many instances other patients can dissuade a potential candidate from an irregular discharge when the staff cannot seem to reach him.

The foregoing measures generally are expensive in staff time and not as yet sufficiently specific to forestall every irregular discharge. Since irregular discharges commonly occur as an impulsive protest against some pressure felt by the individual to be intolerable and since the prediction of such gestures is far from 100 per cent effective, another type of solution has been devised. A specific procedure has been instituted at this hospital in which all patients seeking an against-medical-advice type of irregular discharge must be "cleared" through a special ward. There is a rather laborious routine of transfer to the discharge ward and a "last medical check-up," which must be carried through before the patient can finally obtain his clothes, money, et cetera. At every step along the way, all of the resources of the hospital are brought to bear to slow things down and to solve the immediate problem. The patient is removed from the area of conflict; he has an opportunity to ventilate to "unprejudiced" personnel. Numerous excuses to remain are also afforded him and, if he accepts these, he can do so and still "save face." Staff time, in the form of special orientation and education, case work and psychotherapeutic measures, is economically exerted during this period. Results of this "shotgun" method during its first six months of operation have been quite promising.[5]

Lastly, a very worthwhile (though subtle) outgrowth of weekly irregular discharge conferences has been the modification of staff attitudes and the solidification of staff understanding toward all patients and toward each other. We feel that this is a procedure worth recommending to all tuberculosis hospitals. Each staff member who has direct contact with the patient should attend and participate. In our hospital, the leadership of these conferences has been by the members of the Psychosomatic Section, but this could as easily have been delegated to the ward physician, hospital manager, or caseworker, for example, or to any person interested and moderately familiar with group techniques.

During the past six months, the incidence of irregular discharge has shown a statistically significant decrease at the Madison VA hospital. We are aware that there are considerable spontaneous fluctuations in irregular discharge rates and

[5] Unpublished data.

also that the task of proving the relationship between any particular measure and decrease in irregular discharge requires considerable time and testing. Nonetheless, it seems to us there is little doubt that irregular discharge can be reduced materially by techniques along the lines described above.

It should be recognized that, as there is success in keeping disturbed patients in the hospital, the ward "temperature" rises to a point which sometimes becomes "too hot to handle." Potential irregular discharge patients are "trouble-makers" for the staff. Dealing with them demands much understanding, time, and effort. They place a great strain upon patient-group stability and staff-group stability. The "healthier" the staff, the more this factor can be absorbed. It is theoretically possible to prevent or circumvent *every* irregular discharge. It is practically possible to reduce irregular discharges only within the limits imposed by imperfect human beings organized imperfectly into a hospital staff. The attack upon irregular discharge, therefore, must include further study, not only of irregular discharge patients but of irregular discharge situations in which the hospital *staff* plays a crucial role.

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Notes

THE GEOGRAPHIC DISTRIBUTION OF SARCOIDOSIS

In 1950, Michael and his associates[1] reported a study of the geographic distribution of 226 patients with sarcoidosis who were part of a group of 350 cases observed in military personnel during World War II. An overwhelming majority of these patients had come from the southeastern part of the United States. As this peculiar geographic distribution of sarcoidosis seemed to give a clue to the etiology of the disease, the present writers have attempted to examine their own material from this standpoint.

Accordingly, the records of the Mayo Clinic for the years 1940 through 1951 were reviewed, and 194 cases in which there was histologic evidence of sarcoidosis were found. The age, sex, and race distribution of these patients has already been published in a paper on the prognosis of this disease.[2]

The birthplaces of 177 of these patients were determined from clinic records and by correspondence. The geographic distribution of these birthplaces was then compared with the distribution of the residences of the total clinic registration by groups of states (table 1). There was no indication that sarcoidosis was more common in patients who came to the Mayo Clinic from the southeastern part of the United States than in those who came from other areas.

These data do not confirm the report of Michael and his associates that sarcoidosis is more common in people born in the southeastern part of the United States than in those born in other parts. Instead, the present data indicate that sarcoidosis occurs throughout the United States without any characteristic geographic predilection.

The possibility that many of these patients with sarcoidosis might have lived some part of their lives in the southeastern states was considered, and complete records of their residences were obtained from 136 of these patients by correspondence. Analysis of these residences gave essentially the same geographic distribution as analysis of the birthplaces.

The age, sex, and race distribution of the two groups was considered a possible explanation for the discrepancy. As relatively few Negro patients were in the present series, the data were compared with Michael's data for white inductees without resolving the difference between the two series with respect to geographic distribution. A similar analysis of the present data limited for males aged eighteen through fifty likewise did not provide any explanation of the difference between the two series. Consequently, it is not believed that the discrepancy is due to any of these factors.

It was suggested that the difference in geographic distribution might be due to the inclusion of patients with minimal evidence of sarcoidosis in the present series. An analysis of the birthplaces of 119 of the patients who had disseminated disease revealed the same distribution as did the study of the entire series. Therefore, the difference is probably not due to type of case included in the two groups of cases.

As the difference in geographic distribution of the present series and that of Michael's and associates remains unexplained, the present data are reported so that other series of cases will be similarly analyzed.

In summary: An analysis of the birthplaces of 177 patients diagnosed as having sar-

[1] Michael, M., Jr., Cole, R. M., Beeson, P. B., and Olson, B. J.: Am. Rev. Tubere., 1950, *62*, 403.

[2] Carr, D. T., and Gage, R. P.: Am. Rev. Tubere., 1954, *69*, 78.

TABLE 1

GEOGRAPHIC DISTRIBUTION OF BIRTHPLACES OF PATIENTS WITH SARCOIDOSIS

Geographic Location*	Patients with Sarcoidosis		Total Mayo Clinic Patients
	Number	Per Cent	Per Cent
New England States	0	—	0.2
Middle Atlantic States	11	6.2	2.0
South Atlantic States	4	2.3	2.6
East North Central States	55	31.1	24.6
West North Central States	67	37.9	53.1
East South Central States	5	2.8	1.5
West South Central States	11	6.2	4.0
Mountain States	6	3.4	4.9
Pacific States	4	2.3	2.7
Foreign and Canada	14	7.8	4.4
Total	177	100.0	100.0

* New England—Maine, N. H., Vt., Mass., R. I., Conn.; Middle Atlantic—N. Y., N. J., Penn.; South Atlantic—Del., Md., D. C., Va., W. Va., N. C., S. C., Ga., Fla.; East North Central—Ohio, Ind., Ill., Mich., Wis.; West North Central—Minn., Iowa, Mo., N. D., S. D., Nebr., Kans.; East South Central—Ky., Tenn., Ala., Miss.; West South Central—Ark., La., Okla., Tex.; Mountain—Mont., Idaho, Wyo., Colo., N. M., Ariz., Utah, Nev.; Pacific—Wash., Ore., Calif.

coidosis at the Mayo Clinic during the years 1940 through 1951 revealed the same geographic distribution as that of the total clinic registration. Consequently, it was not possible to confirm the report of Michael and his associates that the disease is more common in people from the southeastern part of the United States.

The difference between the two series does not seem to be due to age, sex, race, severity of disease, or residence other than the birthplace.

SECTIONS OF MEDICINE AND BIOMETRY AND
MEDICAL STATISTICS
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June 23, 1954

AGE DISTRIBUTION OF TUBERCULOUS PLEURAL EFFUSIONS

Tuberculous pleural effusion without demonstrable pulmonary involvement was considered a disease of young persons until a few years ago[1]. Recent clinical observations[2] indicate a shift of its occurrence to older age groups. In order to evaluate the significance of this change, a review has been made of all cases of tuberculous pleural effusion reported to the Health Department of the City of New York in 1950.

Tuberculous pleural effusion is a reportable disease in New York City. Ninety-eight such cases were reported in patients more than ten years of age in 1950. Patients less than ten years of age were not included in the study because, in the age group zero to nine years, it is frequently reported as primary tuberculous infection. The reported data in this age group are therefore not reliable as to the incidence of tuberculous pleural effusion. The 98 cases were almost exclusively reported by general hospitals, since most patients with pleural effusion are usually admitted to a general hospital in this area before being admitted to a sanatorium. Thirty-three of the 98 cases were excluded from the study because of coexisting extrapleural tuberculosis or insufficient evidence for a tuberculous etiology. In the remaining 65 cases, the diagnosis of tuberculous pleural effusion was based on at least two of the following criteria: positive tuberculin test; demonstration of tubercle bacilli in chest fluid, sputum, or urine during the acute illness or subsequent observation period; sterile chest fluid with a preponderance of lymphocytes; demonstration of tuberculous disease within three years after the pleural effusion.

TABLE 1

AGE AND SEX DISTRIBUTION OF TUBERCULOUS PLEURAL EFFUSIONS

Age in Years	Number of Patients	
	Male	Female
10–19	5	5
20–29	10	16
30–39	11	4
More than 40	11*	3†
Total	37	28

* 9 more than 45 years of age.

† 2 more than 45 years of age.

The age and sex distribution of the 65 cases are given in table 1. Eleven (29 per cent) of 37 tuberculous pleural effusions in male patients occurred after the age of forty; the corresponding figures for female patients are 3 (17 per cent) of 28 cases. From the clinical viewpoint, the findings suggest that a tuberculous etiology of a pleural effusion will have to be considered with increasing frequency in male patients more than forty years of age. From the epidemiologic viewpoint, the findings may raise the question of primary infection occurring in male persons more than forty years of age if Wallgren's statement[3] that

[1] MYERS, J. A., AND MCKINLAY, C. A.: The Chest and the Heart, Charles C Thomas, Springfield, Illinois, 1948.

[2] ROBERTSON, R. F.: Brit. M. J., 1952, No. 4750, 133.

[3] WALLGREN, A.: Tubercle, 1948, *29*, 11, 245.

tuberculous effusion usually follows by three to seven months after primary infection holds true for adults as well as for children.

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A CARD FOR INDEXING CLINICAL DATA ON PATIENTS IN A TUBERCULOSIS HOSPITAL

Physicians in hospitals devoted to the treatment of tuberculosis face the problem of indexing large amounts of clinical data for ready future reference. Lack of professional time and limited or unskilled help in the record room make the chore excessively arduous. Card indexes for single diagnoses, drugs, and operations are not always satisfactory when files are searched for records of patients with certain combinations of clinical findings and therapeutic regimens.

This article is concerned with a brief description of a form, designed by the present writer, for indexing clinical data from each patient on a Keysort[®][1] card. The card is simple to use and should be adaptable to hospitals of any size. It permits rapid identification of cases with interrelated social, diagnostic, and therapeutic backgrounds. Data for as many as five admissions of each patient can be indexed on a single card. More than 150 items are listed, 91 of which are keyed in the perforated margin of the card; 131 items require no more than a check mark, and the remaining items require no more than a number or a brief word. Only a fraction of these entries are necessary for any given patient.

General Description of the Card

Clinical data for each patient are registered on both sides of the card, which measures 5 by 8 inches (figures 1 and 2). The numbers 1, 2, 3, 4, and 5 over the boxed columns refer to admissions. If the patient has had more than five admissions to the hospital, the numbers of subsequent admissions are superimposed on a second card and reference is made to the second card in the upper right corner of the face of the first card (such as, "See card No. 2"). If more than one card is needed for a patient, the margins of each card are punched alike and the cards are riveted together. They may then be swiveled apart for inspection.

Each margin of the card is designated by a letter. As one looks at the face of the card, the left margin is *A*, the upper margin is *B*, the lower margin is *C*, and the right margin is *D*. The marginal key for selected items on the face of the card is restricted to margins *A*, *B*, and the first six perforations of margin *C*. The marginal key for selected items on the reverse side of the card is restricted to the balance of margin *C* and to margin *D*.

To facilitate the keying of selected data in the margins of the card, as many items as possible are already identified by margin and perforation number. For example:

A 6—Male patient
A 14—Readmission because of roentgenographic or bacteriologic relapse
B 19—Tuberculosis of trachea or bronchus
C 5—Nontuberculous urogenital or endocrine disease
C 7—Chemotherapy administered
C 22—Lobectomy
D 16—Sputum "converted to negative" (number of months entered).

Some Details About the Face of the Card

Nontuberculous diseases are checked under "Non-TB Disease." These are identified by systems in accord with the *Standard Nomenclature of Diseases and Operations*,[2] as

[1] Keysort® cards are a product of The McBee Company, 295 Madison Avenue, New York 17, New York.

[2] PLUNKETT, R. J., AND HAYDEN, A. C.: Standard Nomenclature of Diseases and Operations, ed. 4, The Blakiston Company, Philadelphia, 1952.

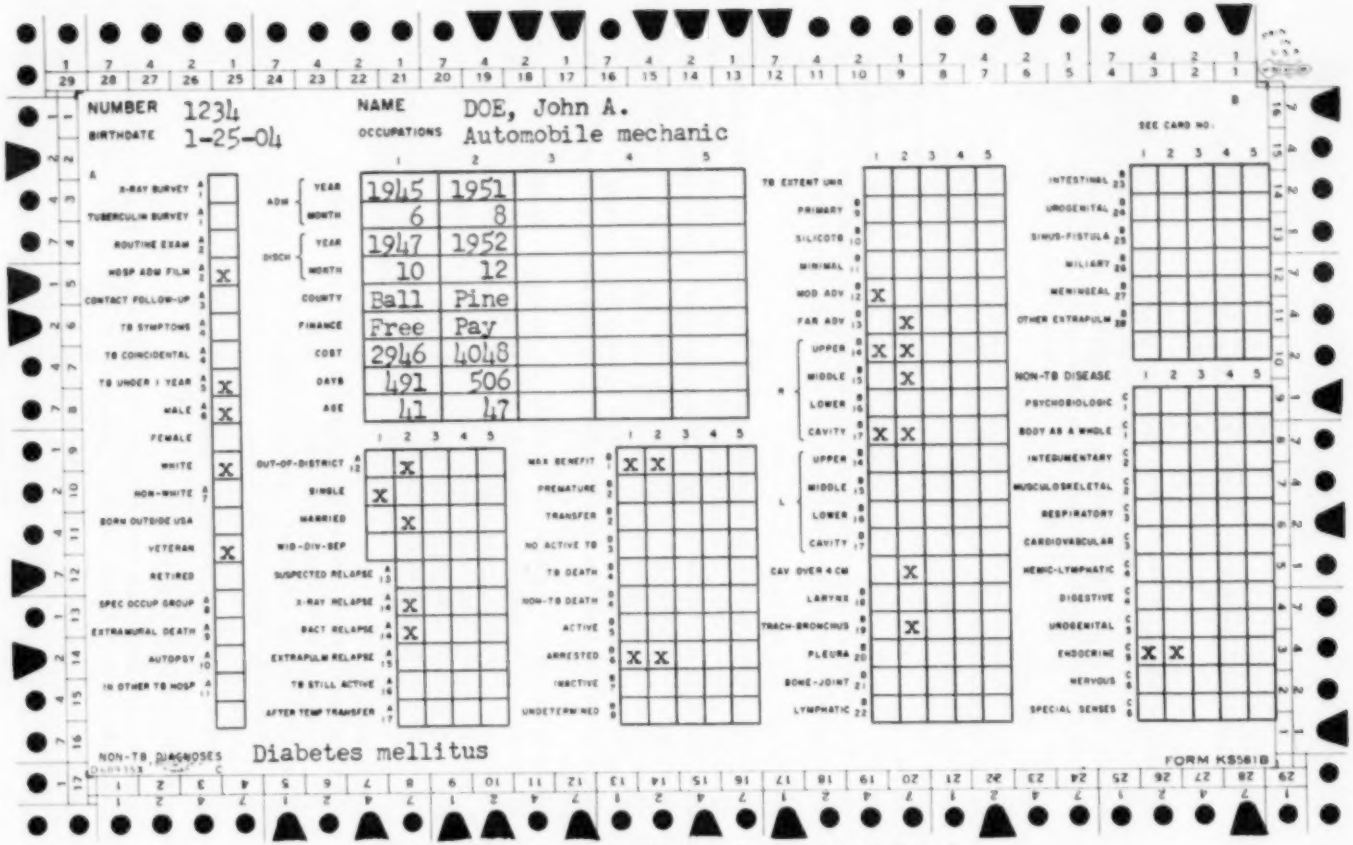

FIG. 1. The index card, with entries for a hypothetic patient

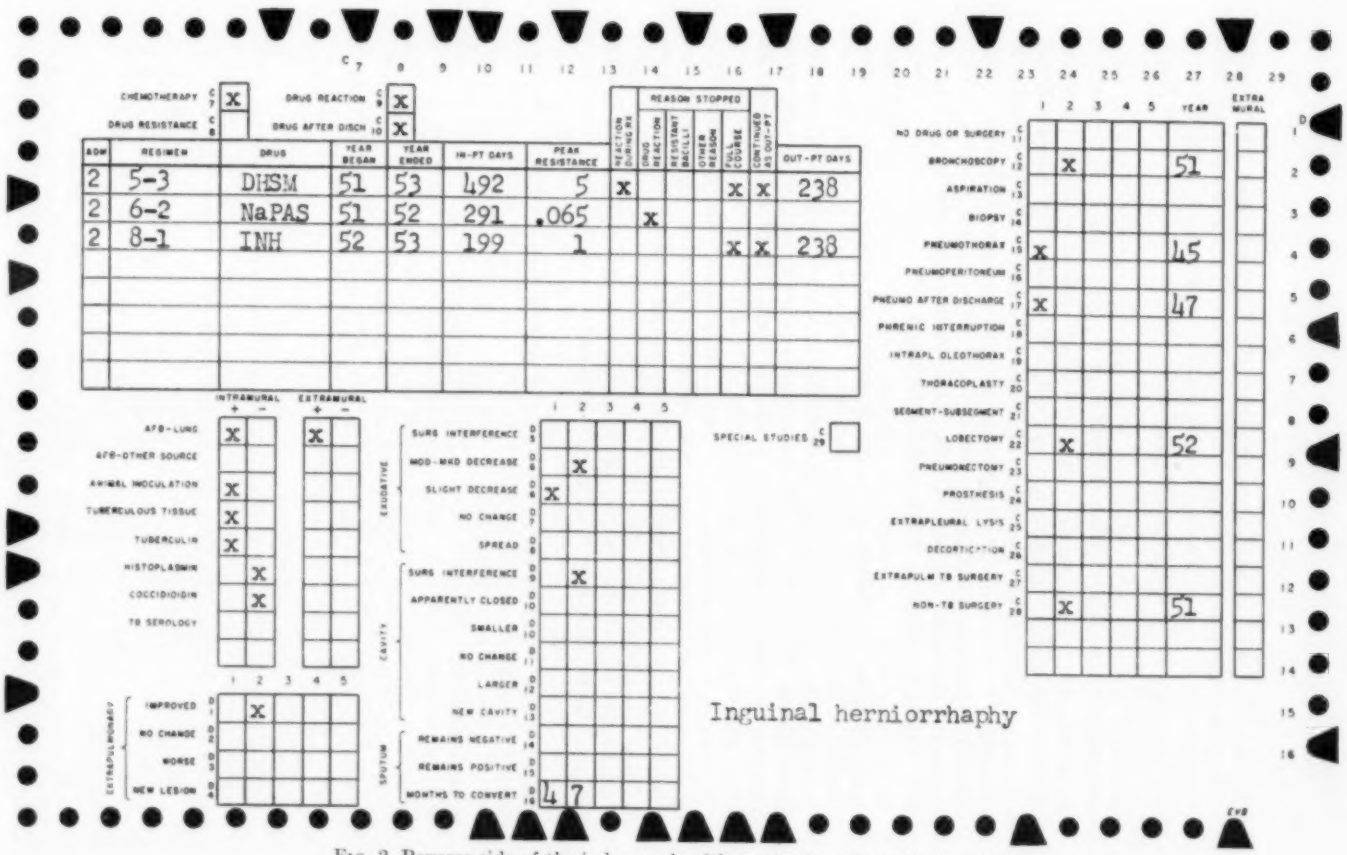

FIG. 2. Reverse side of the index card, with entries for a hypothetic patient

recommended by the American Medical Association. The specific diagnosis is recorded by a word or two in the space at the bottom of the card after "Non-TB Diagnoses." Items *B* 14, 15, and 16 may refer to upper, middle, and lower thirds of the lung fields, or to lobes of the lungs. In the latter case, "middle" of the left lung would refer to the lingula of the left upper lobe.

Some Details About the Reverse Side of the Card

Under "Regimen," drugs and their administration are recorded by arbitrary numbers, such as 5-3. For example, dihydrostreptomycin might be assigned the number 5. Its administration in amounts of 1 gm. intramuscularly twice weekly might be identified by the number 3. For rapid reference, the initials of the drug are entered under "Drug," such as DHSM for dihydrostreptomycin.

Laboratory studies and surgical procedures performed in other hospitals or clinics are noted by marking appropriate boxes under "Extramural."

In the lower corner is a space for naming operations performed for extrapulmonary tuberculosis or for nontuberculous conditions, such as arthrodesis of the knee, appendectomy, and herniorrhaphy.

In the space below "Special Studies," arbitrary numbers are entered to identify patients included in various investigations, such as pilot studies on new drugs or regimens, bronchospirometry, and ballistocardiography, or to identify patients who had less common procedures, such as bilateral pulmonary resection.

No index card can be expected to satisfy all needs of all hospitals for tuberculosis. The card described in this article is a useful device for providing concise, comprehensive data with a minimum of time and effort. By its use, clinical and administrative data are readily obtainable for correlation and analysis.

In summary: A card is described for indexing a large amount of clinical data for patients with one or more admissions to a tuberculosis hospital.

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April 26, 1954

INFLUENCE OF TUBERCULOSIS ON THE METHYLENE BLUE REDUCTION TIME OF SERUM AND HEAT COAGULATION VALUE OF PLASMA

In reporting results of studies on the methylene blue reduction time of serum and heat coagulation value of plasma in cases of neoplastic disease Black[1, 2], Black and Speer[3], Stettner and associates[4] contended that, within limits, these tests were of aid in detecting cancer, but commented that false positive results were found in tuberculosis, cirrhosis, pregnancy, and acute rheumatic fever. As any procedure which aids in differentiating tuberculosis from neoplastic disease is of particular interest to the staff of this institution, a grant from the Ontario Cancer Treatment and Research Foundation was sought and obtained to investigate whether or not the disturbance of the tests in tuberculosis was common enough and of sufficient extent to interfere seriously with their otherwise alleged usefulness in cancer diagnosis.

As test for the presence of malignant tumors, the two procedures referred to and all other blood tests for cancer have never gained much acceptance[5]. The present writers were not in a position to make an investigation to confirm or deny the contentions of Black and others in this respect. It was possible, however, to settle the question of what influence tuberculosis might be expected to play in affecting the results. The purpose of this note is to record the findings, as they might at some time be of interest to phthisiologists in an entirely different connection.

To establish the normal methylene blue reduction range with our materials and technique, the sera of 1,000 blood bank donors were obtained through the courtesy of the Hamilton Red Cross Transfusion Service. The specimens represented the 3 or 4 ml. of blood left in the bleeding tube after the technician had removed all required for the blood bank. These samples were brought to the laboratory and tested within three hours. The procedure for performing the test was almost identical with that described by Black. In table 1 may be seen the frequency distribution of the results. No serum required longer than twelve minutes for reduction.

The sera of 1,000 tuberculous inpatients receiving various treatments, including streptomycin with para-aminosalicylic acid (PAS), or isoniazid with PAS or streptomycin, were also tested (again within three hours of taking the specimens). The frequency distribution of the values obtained is shown in table 2. Nineteen of the 1,000 patients had sera which required more than twelve minutes for reduction. This confirms, therefore, the observation that sera from tuberculous patients in a sanatorium and under treatment may have prolonged methylene blue reduction times; but the percentage of such cases is less than 2 per cent.

To obtain the normal heat coagulation range with the particular colorimeter and technique used, the plasmas of 250 new and apparently healthy employees were studied. As anticoagulant, the usual 6 mg. of ammonium and 4 mg. of potassium oxalate per 5 ml. of blood were used. The test was conducted exactly as outlined by Black. The

[1] Black, M. M.: Dis. of Chest, 1949, *16*, 169.

[2] Black, M. M.: Rev. Gastroenterol., 1950, *17*, 481.

[3] Black, M. M., and Speer, F. D.: Am. J. Clin. Path., 1950, *20*, 446.

[4] Stettner, M. M., Baron, H., Lowenstein, B. E., Greenwald, A. E., Mersheimer, W. L., and Kleiner, I. S.: J. Lab. & Clin. Med., 1950, *35*, 136.

[5] Russell, W. O., Clark, R. L., Dunn, J. E., Rhoads, C. P., Stowell, R. E., and Westmoreland, M. G.: Am. J. Clin. Path., 1951, *21*, 764.

TABLE 1

FREQUENCY DISTRIBUTION TABLE OF METHYLENE BLUE REDUCTION TIMES OF SERA FROM 1,000 BLOOD BANK DONORS

Reduction Time in Minutes	Number of Cases	Reduction Time in Minutes	Number of Cases
5	1	9	11
5½	19	9½	5
6	173	10	4
6½	208	10½	4
7	286	11	1
7½	183	11½	0
8	88	12	1
8½	16		

TABLE 2

FREQUENCY DISTRIBUTION TABLE OF METHYLENE BLUE REDUCTION TIMES OF THE SERA FROM 1,000 TUBERCULOUS PATIENTS

Minutes	Number of Cases	Minutes	Number of Cases	Minutes	Number of Cases
5	4	9	67	13	3
5½	10	9½	13	13½	6
6	82	10	12	14	1
6½	105	10½	8	14½	0
7	241	11	8	15	0
7½	176	11½	1	15½	0
8	164	12	3	16	2
8½	87	12½	1	16½	1
				17	5

TABLE 3

FREQUENCY DISTRIBUTION TABLE OF HEAT COAGULATION VALUES OF SERA FROM 250 NEW EMPLOYEES

Coagulation Range	Number of Cases	Coagulation Range	Number of Cases
0–5	10	26–30	31
6–10	28	31–35	9
11–15	51	36–40	2
16–20	64	41–45	1
21–25	53	46–50	1

frequency distribution of the normal values appears in table 3. No employee had plasma with a turbidity value of more than 50.

The plasmas of 1,000 tuberculous inpatients, many of whom were receiving streptomycin-PAS or isoniazid-streptomycin or isoniazid-PAS, were also subjected to the test. The frequency distribution of the heat coagulation values in this group is shown in table 4. Fifty-five of the 1,000 tuberculous patients had plasma which gave heat coagulation values of greater than 50. This confirms, therefore, that plasma of tuberculous patients in a sanatorium under treatment may have elevated heat coagulation values; but the percentage of persons who have such plasma is less than 6 per cent.

TABLE 4

FREQUENCY DISTRIBUTION TABLE OF HEAT COAGULATION VALUES OF SERA FROM 1,000 TUBERCULOUS PATIENTS

Coagulation Range	Number of Cases	Coagulation Range	Number of Cases
0–5	29	46–50	35
6–10	83	51–55	33
11–15	110	56–60	8
16–20	157	61–65	6
21–25	147	66–70	1
26–30	133	71–75	5
31–35	118	76–80	1
36–40	81	81–85	1
41–45	52		

With regard to the tuberculous patients who have elevated values for either test, the following points were observed: (*1*) The number on antimicrobial treatment roughly equaled those not so treated. (*2*) Nearly all had far advanced disease; none had minimal disease. (*3*) Although not all of the cases recorded for the one test were also studied by the other test, two-thirds of them were. Yet there was but a single instance in which a patient was found to have both an elevated methylene blue reduction time and an elevated heat coagulation value.

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GROWTH OF MYCOBACTERIUM TUBERCULOSIS FROM RESECTED SPECIMENS UNDER VARIOUS ATMOSPHERIC CONDITIONS

The great increase in resectional surgery and the use of specific drugs in the treatment of tuberculosis in recent years has made the bacteriologist acutely conscious of the "nonviable" organisms frequently encountered in the resected specimens. These organisms may be abundant and typical in appearance on stained smears from resected lesions but may fail to grow in culture or guinea pig. The question of potential viability of these bacilli has been the subject of numerous discussions and experiments.

As it is not possible at will to reproduce *in vitro* conditions analogous to those in the human pulmonary lesion, we are at a disadvantage with respect to determining physiologic characteristics of these organisms. For this reason, experimental studies of the "nonviable" tubercle bacilli should be performed directly on the resected material with the aim of proving or disproving postulated explanations for their inability to reproduce in cultures. In the present communication, it is desired to report, briefly, a set of experiments which were designed to test, in part, such a postulate, and which yielded negative results.

This laboratory has been studying the bacteriology of resected pulmonary specimens in cooperation with the Veterans Administration-Army-Navy study on the chemotherapy of tuberculosis. Specimens which are positive for acid-fast bacilli on microscopy and negative for *M. tuberculosis* on culture have been encountered in this hospital. Accordingly, the possibility was examined that tubercle bacilli in slowly "blocking" lesions might have adopted modified gaseous tolerances and requirements.

In addition to the routine cultures on Löwenstein-Jensen and Petragnani media, each specimen was cultured on Löwenstein-Jensen medium under the following three atmospheric modifications: (*a*) dilution of air with nitrogen to yield only 5 per cent oxygen, (*b*) dilution of air with oxygen to yield 50 per cent oxygen and (*c*) dilution of air with carbon dioxide to yield 20 per cent carbon dioxide and 16 per cent oxygen.

Forty-four lesions from 28 resections were examined in this manner. In general, the gaseous modifications gave the same cultural results as did the control specimens maintained in air. The increased oxygen caused slightly more luxuriant growth, but did not appear to yield a significantly larger number of positive cultures. Although an occasional single colony of *M. tuberculosis* would occur in one of the special gas tubes, while the control culture was negative, the converse was also true and could be explained on the basis of chance distribution of a very few viable bacilli in the inoculum.

The results of these limited experiments do not preclude the possibility that alteration of gaseous requirements has occurred in the bacilli with the lesions. Indeed, Schaefer and associates[1] have reported isolation of a tubercle bacillus requiring 5 per cent carbon dioxide from a sputum specimen. According to Soltys[2], Griffith also isolated a tubercle bacillus with a high carbon dioxide requirement. On the other hand, the broad range of atmospheric compositions employed here on a number of direct clinical specimens from anatomically defined sources confirms the flexibility of gaseous requirements established elsewhere for subcultures and stock strains of tubercle bacilli. For this reason, rigidity of gas tolerance and/or requirement of tubercle bacilli in old lesions is not likely to be the

[1] Schaefer, W. B., *et al.*: Bact. Proc., 1954, p. 94.

[2] Soltys, M. A., *et al.*: Tubercle Bacillus and Laboratory Methods in Tuberculosis, E. & S. Livingston Ltd., Edinburgh and London, 1952, p. 24.

principal explanation for the nonviability phenomenon encountered in some of these lesions.

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A COMPARATIVE STUDY IN THE ISOLATION OF MYCOBACTERIUM TUBERCULOSIS ON ARTIFICIAL MEDIA AND EMBRYONATED EGGS[1]

Although many media and methods have been described for the cultivation of *Mycobacterium tuberculosis*, there is no over-all agreement as to which of them is best for the isolation of this organism under routine diagnostic conditions. Tarshis and Frisch[2, 3] found that media containing blood were equal or even superior to the standard egg media for growth of tubercle bacilli. Similar results were obtained by other investigators.[4, 5, 6]

According to the above reports, cultural methods of establishing the colonies of the tubercle bacilli in pathologic materials usually require several weeks for completion. In 1949, McNelly and Riddell[7] stated that they were able to isolate tubercle bacilli within four to seven days from sputum inoculated into embryonated eggs. As a result of their studies, they suggest that it is possible to use embryonated eggs as a routine procedure for rapid diagnosis of tuberculosis. Recently Brueck and Buddingh[8, 9] also demonstrated acid-fast bacilli in the yolk of embryos four to six days after inoculation of materials collected from patients with suspected tuberculous infections. Isolation of *M. tuberculosis* in embryonated eggs, therefore, may take the place of artificial media for rapid routine diagnosis.

Recently a study has been conducted in which concentrated specimens of sputum were planted simultaneously on Löwenstein-Jensen medium[3], penicillin blood agar[3], and inoculated into embryonated eggs in order to compare the value of these media in actual practice.

Sputum specimens consisted of twenty-four-hour samples collected from known tuberculous patients at Laurel Heights Sanatorium[10] and suspected patients in the Grace-New Haven Community Hospital. All specimens were homogenized with 4 per cent sodium hydroxide. After centrifuging, the sediment was neutralized with 6 per cent hydrochloride acid. The concentrated material was resuspended in saline and inoculated into Löwenstein-Jensen medium and penicillin blood agar slants. These tubes were examined every other day. Acid-fast stained smears were made whenever a slight film appeared on the inoculated surface.

To the remainder of the saline suspension, 100 units of penicillin per ml. were added. Embryonated eggs were inoculated by way of the yolk sac and by chorioallantoic membranes. Five to seven days after inoculation, contents from the inoculated yolk sac were subcultured into artificial media. Acid-fast smears of the unconcentrated yolk were made at this time. When the chorioallantoic membrane route was used, acid-fast smears were made directly from the membrane at the point of inoculation and from the nodules when they were present.

A total of 25 sputum specimens were inoculated on artificial media and into embryo-

[1] This study was made possible by a grant from the William Wirt Winchester Fund.

[2] Tarshis, M. S., and Frisch, A. W.: Am. J. Clin. Path., 1951, *21*, 101.

[3] Tarshis, M. S., Kinsella, P. C., and Parker, M. V.: J. Bact., 1953, *66*, 448.

[4] Clark, M. E.: Am. J. Clin. Path., 1953, *23*, 671.

[5] Reinisch, E.: Am. J. Clin. Path., 1953, *23*, 929.

[6] Hosty, T. S., Freeman, B., and Irwin, C.: The Public Health Lab., 1953, *11*, 143.

[7] McNelly, E., and Riddell, W. A.: Am. J. Pub. Health, 1949, *39*, 1463.

[8] Brueck, J. W., and Buddingh, G. J.: Proc. Soc. Exper. Biol. & Med., 1953, *105*, 304.

[9] Buddingh, G. J., and Brueck, J. W.: J. Louisiana State M. Soc., 1953, *105*, 304.

[10] The writers wish to express their appreciation to the laboratory staff of the Laurel Heights Sanatorium for their assistance in making available the sputum specimens.

TABLE 1

COMPARISON OF RATE OF GROWTH OF TUBERCLE BACILLI MICROSCOPICALLY ON LÖWENSTEIN-JENSEN MEDIUM, PENICILLIN BLOOD AGAR, AND EMBRYONATED EGGS

Time of Earliest Appearance of Growth in Days	Löwenstein-Jensen Medium	Penicillin Blood Agar	Embryonated Eggs
5–6	3	5	0
7–8	3	8	2
9–10	0	0	1
11–12	1	0	3
13–14	1	4	5
15–16	0	2	
25	1	1	
Total positive at the time examined	9	20	11
Percentage positive in 25 cases	36	80	44

TABLE 2

COMPARISON OF RATE OF GROWTH OF TUBERCLE BACILLI MACROSCOPICALLY (COLONIES) ON LÖWENSTEIN-JENSEN MEDIUM AND PENICILLIN BLOOD AGAR

Time of Earliest Appearance of Growth in Days	Löwenstein-Jensen Medium	Penicillin Blood Agar
7–9	0	1
10–12	3	4
13–15	2	1
16–18	2	3
19–21	3	3
22–28	3	3
29–35	2	1
Total positive at the time examined	15	16
Percentage positive in 25 cases	60	64

nated eggs. Comparative studies as determined by microscopy of the rate of growth of tubercle bacilli on the artificial media and in embryonated eggs are shown in table 1. It is noted that the earliest appearance of growth on penicillin blood agar was 44 per cent higher than on Löwenstein-Jensen medium, and 36 per cent higher than on embryonated eggs. The comparative studies of the earliest appearance of colonies on the two artificial media are shown in table 2. This indicates that there was no significant difference between these two media. The cumulative data on growth as determined microscopically, presented in figure 1, indicate that the blood medium showed the earliest evidence of growth within seven to eight days, whereas positive results from embryonated eggs were obtained on the thirteenth to fourteenth day.

Although earlier investigators have shown the value of a blood medium for the cultivation of *M. tuberculosis*, they did not note any significant increase in the rate of growth as compared with that on standard egg media. Macroscopically, this study agreed in that there was no significant difference in the rate of growth, although it certainly indicated

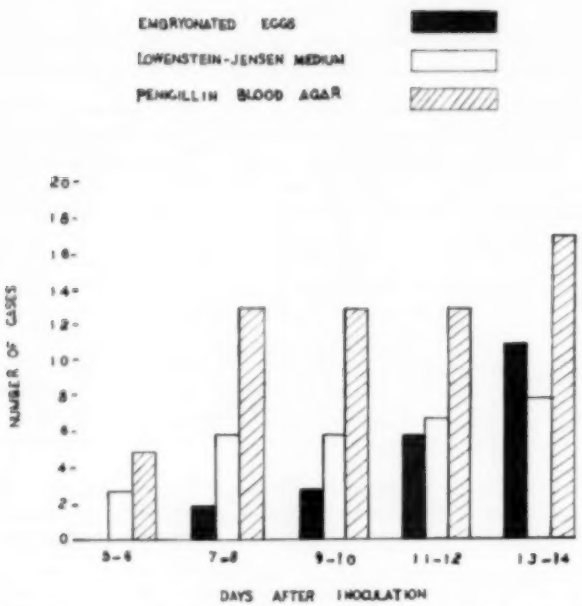

FIG. 1. Comparison of earliest microscopic appearance of growth of tubercle bacilli on Löwenstein-Jensen medium, penicillin blood agar, and embryonated eggs (cumulative result).

that growth appeared earlier on blood medium when examined microscopically. This growth could be overlooked, however, if the observer looked only for colonies, for, as stated before, *M. tuberculosis* grew first as a slight film over the surface of the blood medium. This growth was present within five to seven days and, when acid-fast studies were made, revealed large bundles of organisms, many of which appeared in cord formation. Within two to three weeks colonies could be seen grossly on this medium, which was also the time that they appeared on the Löwenstein-Jensen medium.

Inoculations on the chorioallantoic membrane had the advantage that lesions were evident but required large inoculum. In those instances in which few organisms were present in the inoculum, the nodules usually were not visible within ten days' incubation.

Yolk sac inoculations were simpler to carry out, but, in contrast to the use of the chorioallantoic membrane, it was not possible to detect lesions. Attempts to concentrate the small amount of organisms present in the yolk according to the method of Buddingh and Brueck were unsatisfactory. Further attempts to concentrate the organisms in the yolk with 4 per cent sodium hydroxide followed by extraction with ether and ethylene dichloride also failed.

In those instances in which acid-fast organisms could not be found in the smears of inoculated yolk after five to seven days' incubation, it was noted that subcultures of the yolk directly to artificial media were positive after two to three weeks' incubation. This would indicate that the tubercle bacilli were present in such small numbers that they could not be demonstrated on direct smears.

In summary: For the isolation of tubercle bacilli under routine diagnostic conditions, penicillin blood agar was found to be superior to Löwenstein-Jensen medium in the number of positive cultures obtained as well as in the rate of growth. Early detection of growth on this blood agar can be made only by use of the acid-fast stains, since colony formation does not appear until several days to weeks after primary growth appears. This medium is not difficult to make, is economical, and should, therefore, be used in the laboratory in conjunction with Löwenstein-Jensen medium.

Sputum, in which the tubercle bacilli were present in such small numbers as to give negative acid-fast smears and cultures, was inoculated into yolk sacs of embryonated eggs which were then incubated seven to ten days. Subculture of the yolk was then made on artificial media, with positive cultures becoming apparent in two to three weeks.

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A FILTER PAPER CULTURE TECHNIQUE FOR THE EARLY DETECTION OF MICROCOLONIES OF TUBERCLE BACILLI

There is need for a more rapid detection of *M. tuberculosis* in specimens obtained from patients than is afforded by regular culture methods. The technique here described, although less sensitive than some others, does speed cultural diagnosis. It is simple in comparison with various slide culture and other rapid methods,[1-4] requires no unusual precautions for avoiding contamination, and can be used, within limits, in a routine clinical laboratory.

Sterile 1 by 6 cm. Whatman No. 2 filter paper strips were placed on the surfaces of Löwenstein-Jensen culture slants in screw-top tubes. The cultures were inoculated with suspected materials as sodium hydroxide-treated sputa (with no special precaution other than to run the inocula down the strips) and incubated. After approximately a week the strips were removed aseptically and laid on slides that were previously labeled with Labink, a drop of 5 per cent serum was placed on each strip, the slides were thoroughly fixed by heat and stained by the Ziehl-Neelsen method. The strips generally floated up from the slides, which were favorable for decolorization, but did not fall off the slides unless a too powerful stream of water was used for rinsing. Considerable washing and destaining with acid-ethyl alcohol were necessary to produce the optimal endpoints of faintly colored strips, which proved impossible when cultures were contaminated. Light counterstaining with methylene blue was sometimes performed but appeared unnecessary. The strips were then allowed to dry and were permanently fastened to their slides with Duco cement. The cultures were reincubated for later development of gross colonies, and the strips were saturated with immersion oil (which clarified them considerably) and searched under low power of the microscope for the presence of microcolonies of *M. tuberculosis*, which stained deeply and stood out distinctly when tightly corded (figure 1). Confirmation was made under oil immersion (figure 2) and, occasionally, gentle scraping away of overlying filter paper fibers was necessary to accomplish this. Loosely corded colonies, especially when small, were somewhat harder to detect. At the start 10× oculars were used for searching, but after considerable practice 5× oculars were substituted, which cut the searching time approximately in half. Artifacts, such as irregular coils of red-staining material believed to be mucus, were confusing, but high power showed them not to be *M. tuberculosis* colonies and experience lessened such confusion. Strips from contaminated slants often stained unevenly and deeply and were hard to search, but such strips sometimes showed microcolonies of *M. tuberculosis* while their slants subsequently failed to develop typical gross colonies of acid-fast rods. Oil was removed from the strips with xylol before storing microculture slides. The filter paper strip slants were called "Microcultures" referring to early examination for microcolonies.

Preliminary tests indicated that filter paper strips had no inhibitory effect on the growth of *M. tuberculosis* and showed that a seven-day incubation period allowed a considerable percentage of the viable organisms, which would eventually develop gross colonies, to form identifiable microcolonies. It was also shown that some known acid-fast saprophytes and some presumedly avirulent mycobacteria grew more rapidly on microculture than virulent organisms and formed no cords.

The main work involved two comparisons in which cooperating technicians at Barlow

[1] Pryce, D. M.: J. Path. & Bact., 1941, *53*, 327.
[2] Berry, J. W., and Lowry, H.: Am. Rev. Tubere., 1949, *60*, 51.
[3] Oeding, P.: Acta tubere. Scandinav., 1951, *25*, 208.
[4] Uyeda, S., Yamada, O., and Okada, H.: Acta tubere., Japon., 1951, *1*, 1.

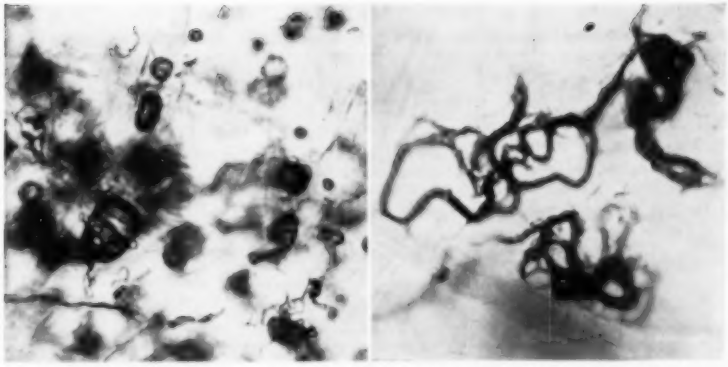

FIG. 1. (Left) Tightly corded *M. tuberculosis* colonies on filter paper. Background is regular filter paper structure, which is much more confusing in black and white than when seen in color. (Low power)

FIG. 2. (Right) A colony from figure 1 under oil immersion

Sanatorium and at the Laboratory of the Tuberculosis Service of the Los Angeles County General Hospital inoculated diagnostic specimens on microculture slants in parallel with their routine cultures. Most of the specimens were sputums shaken with a final 0.75 per cent concentration of sodium hydroxide and not neutralized, although occasional specimens were treated with more sodium hydroxide and neutralized before planting. The microcultures were further processed at the Barlow Sanatorium laboratory, their filter paper strips were examined after incubation, and the slants were then reincubated until at least six weeks after inoculation had elapsed. The slants were examined several times before discard for macroscopic colonies typical for *M. tuberculosis*, and the colonies were checked by examination of a stained smear if they appeared on slants which had yielded negative filter paper strips or if such examination seemed otherwise warranted. At first, only one microculture was inoculated from each specimen and its strip examined after seven days' incubation; later, on the theory that initiation of growth might sometimes be delayed or that some strains of *M. tuberculosis* might grow slowly, two microcultures were usually planted, which permitted the examination of one strip after seven days, the other after ten days. The microculture results and the findings from later incubation of the same slants were compared with the routine culture findings, which were complete after eight weeks. This comparison of one or two microcultures with six routine Barlow or four County Hospital cultures increased the odds in favor of the routine series. The combined results of these two comparisons (Barlow Sanatorium, 342 specimens; County Hospital, 128 specimens) are summarized in table 1.

From table 1 it is obvious that microcultures were not as sensitive as routine methods, especially when read after only seven days' incubation (heading 2). When a second tube permitted an additional microculture reading later than seven days, the sensitivity increased (heading 3). This presumably represents more than the mere addition of a second tube as nine microcultures were positive in ten days and negative in seven days, while the reverse held true in only one instance.

Most of the positive specimens missed by microculture contained relatively small numbers of bacilli; and, in the Barlow comparison, ten of the eighteen specimens in this category showed only one colony of *M. tuberculosis* in six routine culture tubes.

The positive microcultures were scored for their cord formation as follows: no cords—

TABLE 1

COMPARISON OF MICRO- AND ROUTINE CULTURES

1. Total specimens cultured	470
Microcultures Positive After	
2. 7 days' incubation (1 tube)	49
3. 7 and 10 days' incubation (2 tubes)	58
4. Later appearance of gross colonies on slants	69
5. Routine cultures positive	78
6. All cultures positive	85

TABLE 2

Positive Microcultures	Score of Cord Formation	Virulence for Guinea Pigs
4	0	3 nonvirulent
		1 virulent
6	+	2 nonvirulent
		4 virulent
5	++	5 virulent
2	+++	2 virulent

zero; loose, moderately tight, and extremely tight cords—1-plus, 2-plus, 3-plus. Many of the Barlow specimens were routinely inoculated into guinea pigs at the time of culturing. Seventeen such specimens were positive on microculture, and a comparison between their degree of cord formation and virulence for guinea pigs (table 2) is of interest.

Microcultures became contaminated in approximately the same proportion as the routine cultures, but almost all contaminated microculture strips were examined. This was justified by the appearance of many microcolonies on strips from several heavily contaminated slants on which no typical gross colonies later developed.

One unexpected diagnosis was made by microculture when ova of the lung fluke *Paragonimus westermani* adhered to the strips and were beautifully demonstrated by the stain.

The microculture technique is mainly limited for routine use by the time involved in searching the stained strips. Microculture tubes could easily be included with all routine cultures, and this would permit a laboratory to make an early examination of any culture should this seem justified. A microculture tube with the strip left *in situ* is thought to be as prone to develop gross colonies of *M. tuberculosis* as a regular slant. At present, microcultures are being run along with regular cultures on specimens from newly admitted patients at the Barlow Sanatorium.

The colonial structure of acid-fast rods that have grown on microculture strips gives an indication of their virulence. Tight cords probably correspond rather closely to virulent organisms, but loose cords or their entire absence may be harder to interpret. It was observed that cord structure was often by no means uniform and that it could be particularly influenced by contamination.

In summary: A diagnostic microculture technique for *M. tuberculosis* which is practical for limited routine use is described. The technique involves placing sterile filter paper strips on the surface of culture slants and inoculating material for culture directly on these

strips. The strips can be removed, stained, and examined for microcolonies of *M. tuberculosis* after seven or more days' incubation, and the slants can be reincubated for later development of gross colonies. The degree of cord formation of recovered acid-fast rods is well demonstrated by microculture. Microcultures are somewhat less sensitive than routine culture methods but have the advantage of speed, when positive.

Acknowledgments

The authors gratefully acknowledge the cooperation of Grace Hyslop, Anna Moore, and Rose Shimoda at Barlow Sanatorium; of Frances Holtzwart, June Johnson, Mary Epler, and Dorothy Rea at the Los Angeles County General Hospital, for their technical help in this work; and of Lloyd Matlovsky for the photography.

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ASPARAGINASE OF MYCOBACTERIA[1]

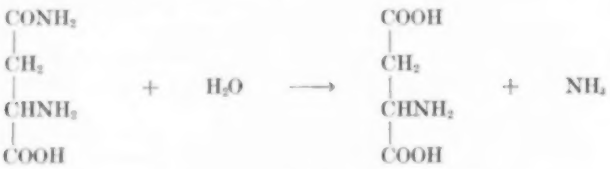
It is well known that the enzyme, asparaginase, is widely distributed among animals, plants, and microorganisms.[2]

Greenstein and Price[3] working with aqueous extracts of rat liver have described a heat-labile asparaginase I and a more heat-stable asparaginase II which is activated by alpha-keto acids. On high-speed centrifugation, all the asparaginase activity was found in the supernatant.

There are as yet no precise data on the occurrence of asparaginase in mycobacteria. Evidence that deamidation of asparagine occurs in growing cultures of mammalian and avian tubercle bacilli has been obtained by Long[4] and Bance.[5] More recently, Sym[6] has alluded to "asparaginase activity" of intact tubercle bacilli.

A study of the distribution of asparaginase among the genus *Mycobacterium* and its cell-free preparation and characterization would be of considerable interest for the following reasons. First, asparagine can serve as the sole nitrogen source for all members of this genus which can be grown in artificial culture. Second, there is a paucity of information on anaerobic mechanisms of mycobacteria. The deamidation catalyzed by asparaginase does not involve the participation of oxygen.

Investigations of mycobacterial metabolism by the present writers showed that washed cells of strains H37Ra and H37Rv of *Mycobacterium tuberculosis* var. *hominis*, strain BCG of *Mycobacterium tuberculosis* var. *bovis*, *Mycobacterium sp.* strain 607, and *Mycobacterium smegmatis* formed ammonia from asparagine without oxygen uptake and also under strictly anaerobic conditions. The ammonia determinations were made by Conways' micro-diffusion method. Manometric measurements showed that there was no evolution of carbon dioxide. Filter paper chromatographic analysis of the contents of the reaction vessel showed that aspartic acid was the only other product of the reaction. From this evidence it was concluded that the organisms catalyzed the deamidation of asparagine according to the following equation:



The above organisms, at a concentration of 1 mg. of bacterial nitrogen in 6 ml. of asparagine solution containing 5 micro moles of asparagine, deamidated the asparagine almost completely in four hours at 37°C. and pH 7.2. *Mycobacterium phlei* under these conditions produced only 0.46 micro moles of ammonia per mg. of bacterial nitrogen. This

[1] This investigation was supported by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

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[4] LONG, E. R.: Am. Rev. Tubere., 1919, *3*, 86.

[5] BANCE, J.: Arch. Inst. Pasteur, Tunis, 1942, *31*, 222.

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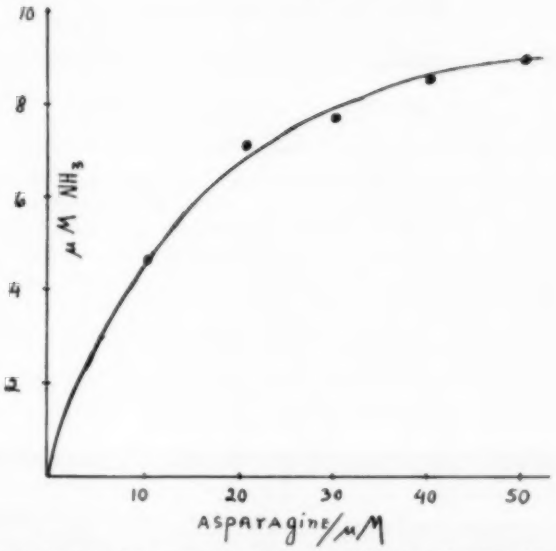

Fig. 1. Effect of asparagine concentration on the velocity of its deamidation by soluble enzyme of *Mycobacterium smegmatis* acting for five minutes at pH 7.5 and 37°C.

organism also grew poorly on a synthetic medium containing asparagine as the sole source of nitrogen. Experiments are underway to elucidate the reason for this exceptional behavior.

The enzyme has been extracted from cells of *M. smegmatis* in the following manner: washed cells are ground in a mortar with 0.1 M phosphate buffer until a suspension of the consistency of a thick paste is obtained. Powdered glass is then added and, following this, the material is milled in a ball-mill at refrigerator temperature for eighteen hours. The cell debris and glass powder are removed by centrifugation. The asparaginase activity is found in the supernatant even after centrifugation at 40,000 r.p.m. for ninety minutes. Substrate concentration has the typical effect on the enzymatic activity. This is shown in figure 1. The enzyme is active at pH 6 to 9.

The enzyme differs from asparaginase I, since it is not inactivated by a temperature of 50°C. for ten minutes and differs from asparaginase II because added pyruvate does not enhance its activity.

Department of Bacteriology
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Waldemar F. Kirchheimer
Charles Keith Whittaker[1]

August 6, 1954

[1] Fellow of the National Foundation for Infantile Paralysis.

Correspondence

TECHNIQUES OF DRUG-RESISTANCE TESTS

To the Editor of The American Review of Tuberculosis:

It is current practice in most laboratories to use massive inocula of tubercle bacilli in the performance of drug-resistance tests *in vitro*. Indeed, it is recommended (American Trudeau Society: Laboratory Subcommittee Report, Am. Rev. Tuberc., 1952, *65*, 105; Medical Research Council: Laboratory Subcommittee of the Tuberculosis Chemotherapy Trials Committee, Lancet, 1953, *2*, 213) that 0.02 to 0.2 ml. aliquots of visibly dense suspensions of bacilli scraped from the surface of egg medium cultures or of bacilli cultivated in Tween®-albumin medium be employed for this purpose. Such are inoculated undiluted onto slants of media containing various concentrations of drugs.

The effect of size of inoculum (in terms of numbers of cultivatable bacilli) on the results of a drug-resistance test is illustrated in figure 1. It is evident from the results that too

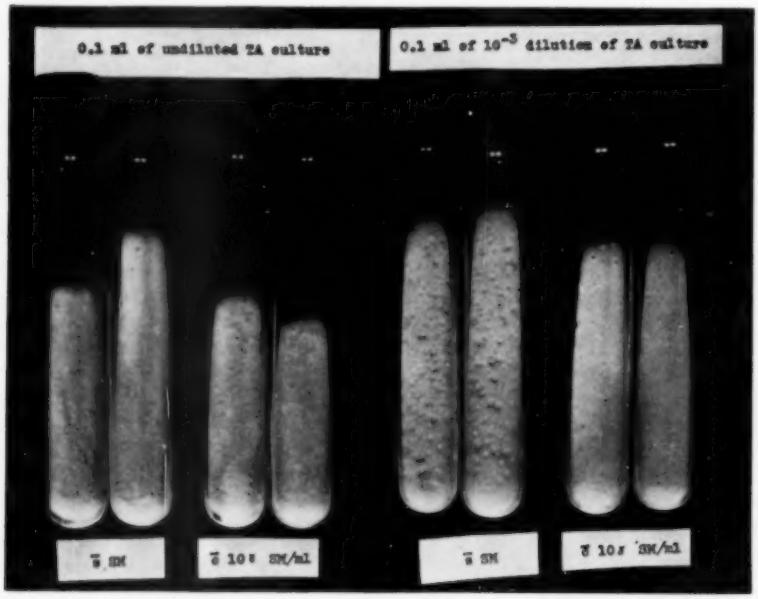

FIG. 1. Masking of drug-susceptible tubercle bacilli by large inoculum in drug-resistance test.

A visibly dense Tween®-albumin culture of bacilli composed of 99.5 per cent of susceptible and 0.5 per cent of resistant organisms was inoculated onto egg-yolk-glycerin-potato (ATS) medium with and without streptomycin.

On the left, 0.1 ml. of the undiluted suspension, and, on the right, the same volume of a thousandfold dilution were used; duplicate tubes were employed.

Confluent and apparently equal growths were obtained on media with and without streptomycin when the large inoculum was used. With the thousandfold diluted inoculum, the actual proportion of susceptible to resistant organisms was brought out.

large an inoculum of living bacilli may easily mislead the bacteriologist into reporting resistance of all or nearly all the individual organisms in the bacterial population tested, when more than 99 per cent of the population may actually be inhibited by the particular concentration of drug used.

Although this arithmetically simple phenomenon has previously been recognized (Wolinsky, E., Reginster, A., and Steenken, W., Jr.: Am. Rev. Tuberc., 1948, *58*, 335; Colwell, C. A., Pitner, G., and Moravec, M.: Am. Rev. Tuberc., 1951, *63*, 679; Tompsett, R.: Am. Rev. Tuberc., 1954, *70*, 91), the fact that no appropriate changes have been recommended in practice (Tuberculosis Laboratory Methods, as revised following the Thirteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, St. Louis, Missouri, February 8–11, 1954, Section I, part 3) prompts us to re-emphasize its importance. Unless and until these simple quantitative considerations are applied in the clinical laboratory, those studies will certainly remain confused which are aimed at answering several clinically important questions, such as the following:

1. What is the correlation between the response to chemotherapy and the drug-susceptibility of tubercle bacilli as determined *in vitro*? Or, at what level of drug-resistance, as determined *in vitro*, may the administration of a drug be expected to be ineffective *in vivo*?

2. How effective are the various possible combinations of chemotherapeutic agents in preventing the emergence of drug-resistant mutants *in vivo*?

3. What is the relationship between isoniazid-resistant tubercle bacilli from isoniazid-treated patients and their attenuation for guinea pigs or other animals?

Sincerely yours,

GARDNER MIDDLEBROOK

DEPARTMENT OF RESEARCH AND LABORATORIES
NATIONAL JEWISH HOSPITAL
DENVER 6, COLORADO

July 23, 1954

TORSION OF THE SPLEEN ASSOCIATED WITH PNEUMOPERITONEUM

To the Editor of The American Review of Tuberculosis:

I was interested in seeing the report by Dr. William H. Falor and Dr. Darrel C. Angus of torsion of the spleen associated with pneumoperitoneum, which appeared in the July issue of THE AMERICAN REVIEW of TUBERCULOSIS.

In order to keep the literature up to date, because I think accurate knowledge of these matters is desirable to practitioners, I would like to report a case of torsion of the spleen which we believed definitely resulted as a complication of pneumoperitoneum occurring in a 28-year-old female in 1953, requiring a splenectomy in January of that year. Clinically, the patient complained of pain in the left upper quadrant, and palpation of that region revealed a rather large, firm mass. Upon surgery, it was noted that the spleen was covered with omentum, with many loops of small intestine adhering to it. The spleen was then found to be twisted on its pedicle in a clockwise manner. A thrombotic splenic vein was discovered. The pathologist's report was chronic congestive splenitis with infarction.

Sincerely yours,

ARTHUR J. VIEHMAN

THE JEFFERSON TUBERCULOSIS SANATORIUM
3800 RIDGEWAY DRIVE
BIRMINGHAM 9, ALABAMA

July 15, 1954

THE USE OF ISONIAZID ALONE IN THE TREATMENT OF PULMONARY TUBERCULOSIS

To the Editor of The American Review of Tuberculosis:

"The Course of Pulmonary Tuberculosis During Long-Term Single-Drug (Isoniazid) Therapy" by Kurt Deuschle, Louise Ormond, DuMont Elmendorf, Jr., Carl Muschenheim, and Walsh McDermott, in the August, 1954, issue of THE AMERICAN REVIEW OF

TABLE 1

DEVELOPMENT OF ISONIAZID RESISTANCE

	2 Months	4 Months	8 Months
Number of patients treated	93	83	53
Sputum culture positive for tubercle bacilli	59	49	41
Susceptible to 0.2 γ per ml.	29 (49)*	13 (27)	3 (7)
Resistant to 0.2 γ per ml.	3 (5)	6 (12)	4 (10)
Resistant to 1.0 γ per ml.	10 (17)	8 (16)	9 (22)
Resistant to 5.0 γ per ml.	17 (29)	22 (45)	25 (61)
Resistant to 1.0 and 5.0 γ per ml.	27 (46)	30 (61)	34 (83)

* The figures in parentheses indicate the per cent of positive cultures.

TABLE 2

COMPARISON OF ROENTGENOGRAPHIC CHANGE WITH RESISTANCE TO ISONIAZID

	Moderate or Marked Improvement		Slight Improvement to No Change		Worse		Total Patients
	Number	Per Cent	Number	Per Cent	Number	Per Cent	
Results at 4 Months of Treatment							
Resistant to 1.0 γ per ml.	2	25	4	50	2	25	8
Resistant to 5.0 γ per ml.	2	9	17	77	3	14	22
Susceptible and negative	20	38	32	60	1	2	53
							83
Resistant to 1.0 and 5.0 γ per ml.	4	13	21	70	5	17	30
Susceptible and negative	20	38	32	60	1	2	53
							83
Results at 8 Months of Treatment							
Resistant to 1.0 γ per ml.	2	22	6	67	1	11	9
Resistant to 5.0 γ per ml.	6	24	11	44	8	32	25
Susceptible and negative	11	58	8	42	0	0	19
							53
Resistant to 1.0 and 5.0 γ per ml.	8	24	17	50	9	26	34
Susceptible and negative	11	58	8	42	0	0	19
							53

TUBERCULOSIS has prompted the re-reporting of some data which was originally contributed to the Quarterly Progress Report of the Veterans Administration, Army, and Navy Study on the Chemotherapy of Tuberculosis, April, 1953, page 45.

Beginning in February, 1952, isoniazid alone in a dosage of 150 mg. daily was given to a number of patients, most of whom were treatment failures after the use of streptomycin and PAS, as well as collapse or excisional procedures, when applicable. Thus good therapeutic results would not ordinarily be expected in such a group, and for this reason they cannot be considered comparable to cases reported by the Medical Research Council of Great Britain, the United States Public Health Service, or the randomized cooperative study of the Veterans Administration. The patients reported below were treated primarily to permit the study of toxicity and the rate of emergence of isoniazid-resistant organisms, and, of course, with the hope that some clinical benefit would also accrue to them.

As may be seen in table 1, seemingly significant resistance to isoniazid occurred rather promptly in a high proportion of patients whose sputum remained positive.

The cases were then tabulated to compare development of resistance to isoniazid with improvement, or lack of it, in the appearance of the disease seen in chest roentgenograms. Preliminary study of the raw data showed that, in this particular group, those who retained susceptible organisms, even though their sputum remained positive, behaved the same as those whose sputum became negative.

For this reason they were combined in the table (table 2).

It is believed that it can be readily concluded that the development of isoniazid resistance was not an innocuous occurrence in that all but one of the patients who showed evidence of advancing disease in chest roentgenograms harbored resistant organisms, while the preponderance of those who did well had susceptible ones, or a negative sputum.

While it is possible that isoniazid-resistant tubercle bacilli may develop less frequently in patients with minimal tuberculosis than in those with more advanced disease, there is also a distinct possibility that this may not be the case. In any event, the point has not been subject to proof either way because of the lack of data in the form of positive sputum cultures in patients with minimal disease who have received isoniazid. It is our belief that isoniazid should not be used alone in the routine treatment of pulmonary tuberculosis, including disease of minimal extent.

Sincerely yours,
WILLIAM S. SCHWARTZ
R. E. MOYER

VETERANS ADMINISTRATION HOSPITAL
OTEEN, NORTH CAROLINA

August 23, 1954

Books

TUBERCULOSE PULMONAIRE ET PLEURALE, Collection Medico-chirurgicale a révision annuelle (Revisions up to 1953). By Pierre-Bourgeois. Pp. 945, Les Éditions Médicales Flammarion, Paris, 1949.

Pulmonary and Pleural Tuberculosis is one volume of a collection of medical texts prepared under the direction of Pasteur Vallery-Radot. Yearly revisions are added to the chapters if new developments call for it.

This is in many respects a remarkable book and presents an almost complete review of the various aspects of pulmonary and pleural tuberculosis such as bacteriology, pathology, symptomatology and diagnosis, course of the disease, and treatment, epidemiology, and prevention. Some of the chapters are written specifically for the French reader, with details on climatic stations in France and the French laws and regulations concerned with the prevention and detection of tuberculosis. The psychological and sociological problems confronting physicians, social workers, and others in the case of tuberculous patients is dealt with in what may be called the typical French way. A combination of great technical skill and high respect for the sick and his individuality can be felt throughout the book. This becomes most obvious in the chapters entitled "La Cure de Repos", "La Prévention de la Tuberculose," and "La Tuberculose Maladie Sociale." The chapter on the use of chemotherapeutic agents is especially well written. The early beginning of specific treatment and the judicious use of combinations of the conventional forms of treatment are emphasized. The fact is stressed that streptomycin or isoniazid have greatly enhanced the success of, and the indication for, collapse therapy. It is also impressive that there were fewer patients left without treatment through the use of chemotherapeutic agents. Although the clinical descriptions are accompanied by schematic illustrations of roentgenographic findings, it is regrettable that they are not further illustrated with pictures of pathologic findings. This would greatly increase the dynamic impression already conveyed by the mere clinical description.

This book is written primarily for the young specialist and the practitioner who encounters cases of tuberculosis in his community, but the experimental worker can well profit from it. To the specialist concerned with laboratory experimentations and theoretical considerations, the clinical orientation of most of the chapters is a revealing insight into the tuberculous infection as seen in humans. The chronicity of the infection and the fluctuations of the host-parasite relationship of the tubercle bacillus and man from symbiosis to parasitism are dealt with. For this reason it is to be regretted that the chapters containing experimental and theoretical material are sharply separated from those dealing with the clinical and epidemiologic aspects of tuberculosis. This lack of integration cannot possibly be compensated by the nearly complete coverage of the material. History and pathology of typical cases rightly provide the central core of the book; these examples should also form the background for the discussion of theoretical problems of the tuberculous infection. Thus, the changes of resistance or susceptibility to the tuberculous infection in a given person with time or the differences of resistance between individuals could serve as an excellent example to discuss almost all aspects of pathogenicity of tuberculosis, such as properties of the tubercle bacillus and native and acquired resistance. Clinical observations as well as experimental results could contribute to an understanding of a problem of such great complexity. The cooperation of the clinician is es-

pecially needed because the human disease offers the best, if not the only, examples of "spontaneous" fluctuations of resistance which no animal experiment can duplicate.

EMANUEL SUTER
Boston, Massachusetts

ANNUAL REVIEW OF MEDICINE, Volume 5. Edited by W. C. Cutting. Pp. 490, Annual Reviews, Inc., Stanford, California, 1954, Price, $7.00.

This volume, the fifth in the series, provides a stimulating approach and at least a partial answer to what the editors refer to as "the insatiable problem of current literature."

The field of internal medicine has been divided, for the purposes of the book, into its various classical disciplines, e.g., infectious disease, cardiology, gastroenterology, hematology, endocrinology, et cetera, and, in addition, allied fields in which new developments are of interest to the internist are covered (dentistry, anesthesia, radioactivity, toxicology, pediatrics, et cetera). The reviews have been written by distinguished clinicians and investigators and reflect, by and large, the special interests of the authors. Since the policy of the editors is to have the various sections written by different men each year, the emphasis on specific topics within the various sub-specialty fields shifts from volume to volume.

Among the articles of particular interests are Jawetz' excellent review of recent work in the infectious diseases, which includes a consideration of the indiscriminate use and harmful effects of antibiotics.

In addition, Newman and Pocock have written a scholarly chapter on kidney disease, with emphasis on the physiologic mechanisms concerned in the regulation of water and sodium excretion.

Other reviews which should be especially mentioned are those of Stefanini and Dameshek on the hemorrhagic disorders, Soffer and Gabrilove on endocrinology, Gellhorn and Holland on medical care in advanced cancer, and Lilienthal and Riley on the pulmonary circulation and gas diffusion in the lung.

The book also contains an excellent annotated list of review articles in medicine, totaling 498, which should prove to be of further help in coping with the volume of the current literature.

All in all, this book will be of value and of interest to clinicians and investigators in internal medicine and allied fields. The Editorial Committee is to be congratulated for having maintained the quality of the series so successfully.

JEREMIAH A. BARONDESS
New York, New York

SYNOPSIS OF MEDICAL ENTOMOLOGY. By V. E. Brown. Pp. 219, Edwards Brothers, Inc., Ann Arbor, Michigan, 1953.

This is a practical work, with a limited objective that has been attained. It seeks to provide medical workers with an elementary knowledge of the morphologic features of the more important insects, especially those involved in the transmission of human disease. Mites, ticks, flies, and mosquitoes are classified simply, described briefly, and illustrated in black and white ink drawings. It would be difficult for a solitary worker in the field to classify an insect on the basis of the material presented but the work should prove useful

if almost any type of entomologic guidance in class, or otherwise, is available. Clinical features of diseases mentioned are omitted. Limited suggestions for insect control are included and the methods of performing a malaria survey are described in some detail. The references are well selected.

The work will be of interest and help to those responsible for the teaching of tropical medicine, public health, and sanitation.

B. H. Kean
New York, New York

BOOKS RECEIVED

Anatomy of the Bronchovascular System. Its Application to Surgery. By George L. Birnbaum. Pp. 300, with illustrations, Year Book Publishers, Inc., Chicago, Illinois, 1954, Price, $15.00.

Antibiotika-Fibel. Indikation und Anwendung der Chemotherapeutika und Antibiotika. By Albert M. Walter and Ludwig Heilmeyer. Pp. 813, Georg Thieme Verlag, Stuttgart, 1954, Price, $18.80.

The Chest. A Handbook of Roentgen Diagnosis. Second Edition, by Leo R. Rigler. Pp. 380 with illustrations. Year Book Publishers, Inc., Chicago, Illinois, 1954, Price, $8.00.

General Cytology. By E. D. P. DeRobertis, W. W. Nowinski, and Francisco A. Saez. Pp. 456 with illustrations, W. B. Saunders Company, Philadelphia, 1954, Price, $7.75.

Ergebnisse der Gesamten Tuberkuloseforschung. By H. Beitzke, St. Engel, L. Heilmeyer, J. Hein, and E. Uehlinger. Pp. 488 with illustrations, Georg Thieme Verlag, Stuttgart, 1954.

The Hidden Causes of Disease. By Antonio Benivieni, translation by Charles Singer (biographical appreciation by Esmond R. Long). Pp. 217, Charles C Thomas, Springfield, Illinois, 1954, Price, $6.75.

Mass BCG Vaccination in Algeria, 1949–1952, with Special Reference to Statistics on Tuberculin Testing and BCG Vaccination. Pp. 49, The International Tuberculosis Campaign, UNICEF, 24 Rue Borghèse, Neuilly-sur-Seine, France.

BCG Vaccination in Finland and the Finnish Vaccination Index. Pp. 41, The International Tuberculosis Campaign, UNICEF, 24 Rue Borghèse, Neuilly-sur-Seine, France.

A Primer of Pulmonary Function. By Harold Guyon Trimble and James Kieran. Pp. 22.

Surgery of the Chest. By Julian Johnson and Charles K. Kirby. Pp. 387 with illustrations, Year Book Publishers, Inc., Chicago, Illinois, Price, $9.00.

The Surgery of Pulmonary Tuberculosis. By James H. Forsee. Pp. 208 with illustrations, Lea & Febiger, Philadelphia, Pa., 1954, Price, $6.50.

BCG and Vole Vaccination. By K. Neville Irvine. Pp. 96, with illustrations, National Association for the Prevention of Tuberculosis, Tavistock House North, Tavistock Square, London, 1954.

Coronary Heart Disease in Young Adults. By Menard M. Gertler, Paul D. White, and others. Pp. 218, Harvard University Press, Cambridge, Massachusetts, 1954, Price, $5.00.

Die BCG-Schutzimpfung. By Rolf Griesbach. Pp. 330, Georg Thieme Verlag, Stuttgart, 1954.

Die Tuberkulosebekampfung in de Schweiz. La Lutte Contre la Tuberculose

EN SUISSE. Publication of the Swiss Tuberculosis Association Against Tuberculosis. Edited by H. Birkhauser. Pp. 374, S. Karger, Basel and New York, 1954.

BRITISH MEDICAL BULLETIN. Tuberculosis, Volume 10, Number 2. Medical Department, The British Council, 65 Davies Street, London, 1954, Price, $2.75.

TRANSACTIONS OF THE THIRTEENTH CONFERENCE ON THE CHEMOTHERAPY OF TUBERCULOSIS. VETERANS ADMINISTRATION-ARMY-NAVY. Prepared and edited by the Veterans Administration Area Medical Office, St. Louis, Missouri, and the Department of Medicine and Surgery, Central Office, Washington 25, D. C. Pp. 442, 1954.

ATLAS OF EXFOLIATIVE CYTOLOGY. By George N. Papanicolaou. Harvard University Press, Cambridge, Massachusetts, 1954.

American Trudeau Society

Medical Section of the National Tuberculosis Association

Changing Concepts and Modern Treatment of Tuberculosis

Report of Panel Discussion Held at the Annual Meeting of the American Trudeau Society, Atlantic City, New Jersey, on May 20, 1954

William B. Tucker, M. D., *Chairman*

Dr. Tucker: A panel discussion entitled "Changing Concepts and Modern Treatment of Tuberculosis," the last event of the medical sessions at this annual meeting, is about to get under way. As a refreshing introduction, I have no announcements to make. The program for this panel as printed is in error in one respect in that the name of one person who was to have participated was omitted. That was Dr. Walsh McDermott. Dr. McDermott's name should have been there, but he is unable to be here and we have asked Dr. D'Esopo to take his place. The members of the panel, therefore, from my left, your right, proceeding to the south are: Dr. Nicholas D'Esopo (well known to all of you), Chief of the Tuberculosis Service at the West Haven Veterans Administration Hospital; next, Dr. Donald L. Paulson, attending physician at Baylor Hospital in Dallas, Texas, and clinical assistant professor of thoracic surgery at the University of Texas Medical School, Southwestern Branch; Dr. Daniel E. Jenkins, Associate Professor of Internal Medicine at Baylor University College of Medicine in Houston, Texas; Dr. Roger S. Mitchell, Clinical Director of Trudeau Sanatorium and Associate Professor of Medicine at the University of Vermont, College of Medicine; Dr. Wendell H. Hall, my associate at the Veterans Administration Hospital in Minneapolis, where he is on the medical service, and Associate Professor of Medicine at the University of Minnesota Medical School; and last of all, to show you the prescience and wisdom of the committee in selecting members for the panel, the 1954 Trudeau Medalist, Dr. Edgar M. Medlar, of Hermann M. Biggs Memorial Hospital, Ithaca, New York.

This panel is going to proceed informally. There has been no "packing" of the panel. We had lunch together and discussed the general subject. We did not agree on any questions, and, in fact, none of the panelists has seen the actual questions in front of me to be directed to them. I'm going to start out by asking Dr. Medlar this question. In general, what were the principal causes of failure of various forms of treatment of pulmonary tuberculosis in the prechemotherapy era, Dr. Medlar?

Dr. Medlar: I would say that, in the prechemotherapy days, the chief reason for failure of treatment of tuberculosis was the fact that necrotic tuberculous lesions sloughed and there was no way to control it. If you could get rid of the process of sloughing, tuberculosis would have disappeared from the map years ago.

Dr. Tucker: Would you say, Dr. Medlar, that this applied to bed rest alone, to collapse therapy, thorocoplasty?

Dr. Medlar: Yes. There are failures in all forms of therapy, as far as I have been able to judge. While you had a decrease in the amount of sloughing in a great many cases and, therefore, in the amount of sputum, the reason for failure was that this process of sloughing became manifest again. The lesions already there caused the relapses.

Dr. Tucker: And to anticipate, Dr. Medlar, some of our later discussion, with the results of some of our more modern, more recent treatment of pulmonary tuberculosis, would you say that the same pathologic facts explain the fewer failures of today's treatment?

Dr. Medlar: I'd like to inject a little thing in here before I answer the chairman's question. When this panel was first discussed, I wrote to Dr. Tucker and told him I didn't see any place in this panel for a pathologist. The place of a pathologist is in the laboratory, and it's his duty to try to bring out the facts, pathological facts. How those facts are used in the care of tuberculous patients is entirely out of the hands of the pathologist and in the hands of the clinician and the surgeon. Now to answer Dr. Tucker's question—as I have studied many hundreds of specimens which have been resected, I have found one disturbing factor. There are two parts: First, all patients with tuberculosis do not react the same to their disease; second, despite the length of chemotherapy, in a certain proportion of these cases there is still evidence of active liquefaction going on in the residual necrotic lesions. I expect as time goes on that it will be this phase of the pathology which will be the stumbling block that all of you will have to face.

Dr. Tucker: Thank you, Dr. Medlar. In reply to the letter that Dr. Medlar is referring to, I told him that we needed an authority, and you have seen in the lay press that a pathologist is a physician's physician. That is the way we look upon Dr. Medlar.

Dr. Medlar: Thank you, Dr. Tucker.

Dr. Tucker: Now, we have talked about failure in very general terms. We merely know that in the past we had failures—today, we have failures. We think that they are less frequent. Dr. Mitchell, do you think that you could give us a very rough estimate of what was the percentage of failure of treatment before the era of chemotherapy?

Dr. Mitchell: There were some statistics published about five years ago indicating that the presence of cavity would mean that 90 per cent of the patients with cavity would be dead within five years. If bilateral cavity were present, about 96 per cent would be dead. This was country-wide. At Trudeau, while the figures are not complete for advanced tuberculosis, it is my guess that, at the end of twenty years, 50 per cent of those with far advanced tuberculosis will be dead of tuberculosis. About 25 per cent of those with moderately advanced tuberculosis on admission will be dead in twenty years, and I already know the final figure on minimal, 5 per cent. Now that is mortality as a measure of the results of treatment. The question of relapse introduces all kinds of variables. If one uses simply loss of time and inability to work, the loss of time caused by minimal tuberculosos has not been very great; not nearly as great as I had thought. I don't know what it is for moderately advanced disease, but I know that the average duration of treatment for moderately and far advanced disease in the cases I am now reviewing is certainly going to average three or four years.

Dr. Tucker: Thank you. That, then, very briefly, is the background against which we will be discussing today's treatment of tuberculosis and the concepts which have been changing from the period referred to. Dr. D'Esopo, in your opinion should all patients with active tuberculosis receive the benefit of antimicrobial therapy?

Dr. D'Esopo: Well that's what we do now. We do treat every case of active tuberculosis.

Dr. Tucker: Don't hedge.

Dr. D'Esopo: We also treat patients who are bacteriologically negative. We make a diagnosis of tuberculosis. The reason why I seem to hedge is that I've been wondering whether, in the future, we might withhold chemotherapy from these very small lesions that we discover on routine surveys. We might simply watch them carefully and treat them when they become positive. That sounds awfully unorthodox now and has nothing to do with your question. The answer to your question is, "Yes."

Dr. Tucker: And would you care to define what you mean and how one would determine whether a case is active and therefore treatable in this sense?

Dr. D'Esopo: Well, if the sputum is "positive" by any method, that's activity; if

there has been X-ray change within a few months, that's activity; and also, in young persons, by which I now mean those thirty or less, we assume that the lesions are active, because we assume that the patients recently acquired those lesions, even if we have no information about the stability on X-ray and even if we have no bacteriologic proof. We may be wrong in that group, but at any rate we do it.

Dr. Tucker: So you are referring to the recency of tuberculin conversion in that group?

Dr. D'Esopo: Well, most of the time we don't know what the tuberculin is.

Dr. Tucker: Then just evidence of recency of the existence of disease?

Dr. D'Esopo: To make it more specific, if we find a case of minimal tuberculosis in a person thirty years of age on a factory survey, we will assume that it is active.

Dr. Tucker: That is a rule of thumb that we can keep in mind. Dr. Jenkins, would you agree that all cases of active tuberculosis should receive the benefit of antimicrobial therapy?

Dr. Jenkins: I would, including active minimal disease.

Dr. Tucker: All right, Dr. Mitchell?

Dr. Mitchell: Yes.

Dr. Tucker: Dr. Hall?

Dr. Hall: Yes.

Dr. Tucker: Does Dr. Medlar care to say anything on that point?

Dr. Medlar: Sure. Understand I'm a pathologist, not a clinician. Therefore, I don't have to accept any responsibility for what I say. I hope the day may come when all minimal cases can be treated without drug and wait until you see which will "jump over the fence" and then start something. All of the data that I know anything about show that at least 70 per cent of minimal cases do very well without anything. So why give them all drug?

Dr. Tucker: But at the present time, Dr. Medlar,—you said "I hope the day will come" —you don't feel that that is yet here?

Dr. Medlar: No, in this time of study, I think anything goes.

Dr. Tucker: All right, sir. Dr. Paulson?

Dr. Paulson: I would agree in general except in the case in which there is a diagnostic problem and you're not sure that it is tuberculosis. In this case I would proceed with a more definite approach, namely, excision of the lesion, because of the possibility of bronchogenic carcinoma in certain age groups.

Dr. Tucker: Thank you very much for bringing that into the picture, Don. Having disposed then, very rapidly, of the current status of what may be the indication for antimicrobial therapy which, briefly, is anything judged by the clinician to be active (although different clinicians may differ in definition), all such cases should receive the benefit of antimicrobial therapy.

The next and very important question is what is the drug regimen of choice today? Dr. Hall, I'll give you first crack at that one.

Dr. Hall: Well, I have the conviction at the moment that it is not wise to give one drug alone; that it is best to give two drugs; and that it is best not to give the two most potent drugs, streptomycin and isoniazid, at the same time. Therefore, it would seem to me that the two best regimens would be: streptomycin–PAS or isoniazid–PAS.

Dr. Tucker: Good. I should have qualified this, of course, by specifying original treatment cases. In re-treatment a totally different problem may be present. Dr. Mitchell, how would you answer the question?

Dr. Mitchell: I would agree with Dr. Hall. The only question that arises is that we know that this new pyrazinamide–isoniazid experiment holds great promise. If patients take

isoniazid–PAS and then fail, the fact that they've had isoniazid may preclude the benefits from pyrazinamide–isoniazid. This is an argument in favor of starting streptomycin–PAS at this time.

Dr. Tucker: So you would lean slightly in that direction, Dan?

Dr. Jenkins: I would favor initial therapy with streptomycin–PAS and would agree otherwise.

Dr. Tucker: Don?

Dr. Paulson: I agree.

Dr. Tucker: Nick?

Dr. D'Esopo: Yes, I would use streptomycin–PAS and keep isoniazid, because so far the three regimens seem equally good. In addition to that, I—no I won't say that.

Dr. Tucker: Now I deliberately anticipated one question which is in front of me with my question to Dr. Cohen at the end of the discussion of the last paper in the previous session. But I would like to give other panelists a crack at the same question.

Would you recommend triple-drug therapy under any circumstances? Dr. Mitchell?

Dr. Mitchell: Yes, I would use triple-drug therapy in re-treatment of patients who had taken one or more drugs in whom we do not have sensitivity tests or in whom we did not wish to wait for their outcome. You might alter it after the sensitivity tests had come through.

Dr. Tucker: Would you ever recommend triple-drug therapy in original treatment patients at the present time?

Dr. Mitchell: No.

Dr. Tucker: O.K. What do you conceive to be the advantages and disadvantages of triple-drug therapy in the original treatment patient?

Dr. Mitchell: Well, I think you are putting all your eggs in one basket when you give all three drugs. We've seen a number of reports—the best one I've seen to date, although it is retrospective, is that of Colonel Weir, that triple-drug therapy has no particular advantage over double-drug therapy.

Dr. Tucker: I would like to inject into this picture the work of Dr. Barclay and Dr. Robert Ebert and their associates at the University of Chicago on an entirely different basis, namely, very careful and meticulous laboratory work on the rate of pick-up by the tubercle bacillus of isoniazid, using C^{14} tagged isoniazid. When either streptomycin *or* PAS is employed with isoniazid, there is an accelerated rate of pick-up of isoniazid; but, when *both* streptomycin and PAS are used along with isoniazid, the rate does not exceed that with either streptomycin or PAS used alone with isoniazid. So here is just one little bit which shows that perhaps potentially there isn't any accentuation of the effect when the third drug is added. But I'd like to ask Dr. Hall to comment on this problem.

Dr. Hall: Well, it would seem to me that the more drugs that one gives to a patient, the more chance there is of side effects; the more chance there is of hypersensitivity. There ought to be some definite advantage to which one can point to justify this increased risk. Thus far I haven't seen it for original treatment patients.

Dr. Tucker: And you don't see anything on theoretical grounds from the standpoint of a bacteriologist where there might be an advantage even theoretically?

Dr. Hall: Oh, I suppose that those who are interested in bacterial genetics might argue that theoretically there would be less chance for mutations toward bacterial resistance; but, in actual fact, it has not been proved clinically.

Dr. Tucker: Thank you. Is there any disagreement among the panelists on this point of triple therapy? Nick?

Dr. D'Esopo: I agree that I wouldn't use it, but by that I don't mean that it shouldn't

continue to be studied. I particularly refer to those very sick patients without previous chemotherapy. We must have an answer to that question. Do these very ill patients profit by triple-drug therapy?

Dr. Tucker: Thank you. We all know that the Public Health Service does have under way such a study under the most meticulous circumstances, and Dr. McLean's question earlier this afternoon was directed at that. Unfortunately we don't have the information, but Dr. Cohen assures us we will. I'd like to ask Dr. Mitchell this question: If two of the three drugs, streptomycin, isoniazid, and PAS, are being administered and it is necessary for any reason to discontinue one of the two, should another drug than one of those three be substituted? Or, should the other drug also be discontinued?

Dr. Mitchell: I feel strongly about this, Bill. I feel that, if you have to stop one during a course of double-drug therapy, you should stop both. My reasons for this are: First if you stop one and you continue the other, there is danger of the development of resistance to the one continued. If you substitute the third drug (we have seen 2 or 3 cases—a very small number of those were patients who received such treatment before coming to Trudeau and who should not have developed resistance on continued combined therapy but who had), I suspect it is a risk although I am not sure why.

Dr. Tucker: Does anyone else have any opinions on this? Dan? Nick?

Dr. D'Esopo: We stop both. I think it is interesting, at least in my own experience, that the patients who develop hypersensitivity when you give them drugs are the ones who are not ill. There seems to be less risk in withholding chemotherapy for several weeks while you find out what drugs they are hypersensitive to and while you go through the job of desensitizing them. We do stop both drugs.

Dr. Tucker: Wendell, what do you think?

Dr. Hall: It's been my experience that, with hypersensitivity to drugs in general, such a patient is frequently hypersensitive to more than one drug. And the best thing one can do when it arises is to stop everything that is not absolutely necessary in the treatment of the patient and, after the side effects have subsided, to add just one agent at a time.

Dr. Tucker: Well, isn't there another question involved here? If this policy just advocated is carried out—and I must confess that this is something which I don't personally practice (we replace one drug for which there appears to be intolerance with another and have not noticed the unfavorable events to which you have referred)—isn't another problem implied here? What is the importance of maintaining continuous therapy, Roger?

Dr. Mitchell: I think it is very important to maintain continuous therapy. Interruptions in therapy have been found to be associated with an abnormally high rate of failure of such therapy. However, when one drug is interrupted, the chance of failure is far greater than when both drugs are interrupted.

Dr. Tucker: In the practical situation, then, if you have intolerance for one drug for any reason, you would discontinue both and consider the fact of discontinuation the lesser evil. Is that right?

Dr. Mitchell: Well, in practice, Bill, we find that, when we have to discontinue drugs for toxicity or hypersensitivity, we can resume them very promptly.

Dr. Tucker: Well, that's an important point and I think that clarifies the discussion to a great extent. This whole question of the desirability of continuity of antimicrobial therapy leads naturally to the next question, and a very difficult one it is indeed. This is the duration of chemotherapy. And, Dr. D'Esopo, would you give us your best estimate of what should be the optimum duration of chemotherapy, relating your answer not necessarily to number of calendar months but to any other reference points, any medical events, and qualifying it as you may wish?

Dr. D'Esopo: I'm sure we don't know the duration of chemotherapy. I think that is the

most important thing we can say about it at the moment. We do know that it is improper to discontinue chemotherapy at a time when cavities are still open and when cultures are "positive," because we have seen resistance develop after that time. It is those patients who do very well, with cavities closed and cultures "negative," who are the problem. We don't know how long to go on after that point has been reached, the point that has been called the target point, that is, when the cavities are closed, the cultures are "negative," and the X-rays are stable. How long after that do you continue drugs? As some of you know, there is a protocol in the Veterans Administration, Army, Navy study to try to answer this question. Now, as to practice, we are on this protocol and we randomize patients according to their birth month. Some of them get six months after their target point, some of them get twelve months. In general, we have a minimum duration of one year of chemotherapy. This is purely arbitrary. We simply feel, and I use the word "feel" in its real sense, that no patient should have less than one year of chemotherapy no matter how favorable everything is; no matter when he "converted," no matter how soon the cavity closed, and that sort of thing.

Dr. Tucker: Well, Nick, you've referred to the fact that we don't know for sure, which is obviously true, and you referred to a study to give us some answers. But you started out being a little more practical than that at one point. How would you advise the physician who is wondering how long to treat after the target point is reached as to how long the therapy should be maintained?

Dr. D'Esopo: Well, if I were expected to give a real precise answer, I would say no less than one year of chemotherapy under any circumstances, and I would say six months after target point.

Dr. Tucker: Ordinarily, resection of residual necrotic lesions after attainment of target point is carried out not too long after that. Some questions are directed toward the duration of chemotherapy after such resection. And so, Nick, would you include that in your answer? How long after resection, or would you still refer to the attainment of target point?

Dr. D'Esopo: Yes, the total duration is irrespective of resection. The resection is an incident in the chemotherapy. If by protocol the patient "comes out" to eighteen months of chemotherapy, we continue it no matter when the resection occurred. However, we have this prejudice, I suppose, that a patient should receive at least four months of chemotherapy after resection. Again I want to emphasize that that is purely arbitrary but, if you do things the same way all the time, you may be able to answer some questions.

Dr. Tucker: Isn't our practice in the absence of an answer aimed at an estimate of the minimum effective practice?

Dr. D'Esopo: Yes. I think the situation resembles somewhat the prolonged chemotherapy of syphilis with arsenic and heavy metals. We knew at that time that it was not right to discontinue chemotherapy when the clinical condition of the patient was favorable, when the serology had changed and the patient had no clinical evidence of syphilis. We knew we must go on after that time. I think we are at that same point now.

Dr. Tucker: Dr. Paulson, how would you answer the question about the duration after resection?

Dr. Paulson: Well, I would agree to a minimum of four months and, depending on the case, a longer period than that, perhaps a much longer period than that. It would depend on whether one believed that he had removed only a major focus, whether there were residual lesions still present, whether there was disease in the other lung, and factors such as that; but I believe a minimum of four to six months of antimicrobial therapy after surgery is correct.

Dr. Tucker: We obviously can't cover all the possible combinations of disease and cir-

cumstances since these are only intended to be general comments as general guides. Dr. Mitchell, anything to add?

Dr. Mitchell: No, I agree with Dr. D'Esopo and Dr. Paulson.

Dr. Tucker: There remain a large group of clinical problems, however, in which this duration of therapy is a totally different problem. The nontarget point problem. Dr. Jenkins, do you have any thoughts on the duration of therapy in a patient with pulmonary tuberculosis which does not reach target point?

Dr. Jenkins: Yes I do. This group of patients who can be described as patients who predominantly have cavity and who have "negative" sputum or who have cavity and who have "positive" sputum should, in my opinion, continue to receive the drugs indefinitely, admittedly alternate combinations of drugs (using resistance studies as a guide). Just as long as either one of those situations persists, that is, cavity and "positive" sputum.

Dr. Tucker: Roger, do you want to speak to that same point?

Dr. Mitchell: As long as they persist plus many months more, don't you mean?

Dr. Tucker: We heard a brief report the other day from Dr. Alfred S. Dooneief of Montefiore Hospital on the indefinite continuation of chemotherapy. I think that that applies to this general problem and is really an expression of what has been said, namely, that we don't really know. We have another category rather loosely defined which is sometimes referred to as the good chronic patient. Ordinarily I have in mind, when that phrase is used, the patient who has little symptomatic evidence of the disease and is not markedly "positive." There is active disease as judged by minor changes in the X-ray, and from time to time, at least, small numbers of tubercle bacilli are discharged. Dr. Hall, how long would you feel that such patients should receive the benefits of antimicrobial therapy?

Dr. Hall: I think that such patients should be treated. I think that we should try to rehabilitate these people and to get them in a state where they are no longer a public health menace. If it should take two to three years to attain bacteriologic negativity, I should not think that would be too long a treatment; and possibly longer than that.

Dr. Tucker: As Dr. Chapman and others reported here this afternoon, we are beginning to accumulate some data on this point. I'd like to mention in this connection that one way of judging the efficacy of different durations of therapy is to study the relapse rate. No matter how one may define that relapse rate, X-ray relapse, bacteriologic relapse, or clinical relapse. And on the question of X-ray relapse we have some rather interesting data from the VA study which have not generally been widely disseminated. In general, it is this: If therapy is maintained for four months (and these are original treatment patients treated with streptomycin and PAS), there is a high relapse rate. There is a continuing X-ray relapse rate during chemotherapy but a fairly slow rate. If therapy is maintained for eight months instead of four and then one determines the relapse rate in later periods, it is definitely less than when therapy is discontinued at four months. If therapy is maintained for twelve months, the relapse rate after the discontinuation of therapy is still less. And if it is maintained for eighteen months, it is still less. The relapse rates after the discontinuation of therapy for comparable periods of observation are decreasingly small the longer the course of the original treatment. And the analysis of data of this type is going to give us some additional answers to the problem. Dr. Hall, do you think the data available on tissue bacteriology bear any significance to the answers to the above questions?

Dr. Hall: Certainly, I think the matter of relapse rate is a very fundamental one. I think that there is every justification for feeling that a patient who has a persistently open cavity as detected clinically is very likely to have bacilli in the lesion which are capable of multiplying in culture and in the inoculated animal. This observation indicates that these

patients are in serious danger of relapse when their chemotherapy is discontinued. Those patients with closed lesions infrequently have bacilli which can be shown to multiply by ordinary culture methods or guinea pig inoculation. I don't feel, however, that this should lead us to believe that such lesions can be disregarded as sources for relapse. Bacilli are there to be seen on smear and, although most of them may be nonviable, so to speak, it is true, I think, that a small proportion of them may still be viable and a source of relapse.

Dr. Tucker: Yes, you are quite right. The question needs to be divided into two parts, the closed lesion and the open lesion. Dr. Medlar, would you like to say anything about the significance of tissue bacteriology under either of those circumstances?

Dr. Medlar: All I can say is that I have never seen a healed open cavity of tuberculosis and, in the specimens I have had a chance to examine bacteriologically, it is extremely rare that you can't get organisms to grow and, if they grow, they are pathogenic. As to the problem of the bacteriology of the closed lesion, which should include filled-in cavity as well as necrotic lesions that have never sloughed out, it is surprising the tremendous numbers of bacilli that one can find unexpectedly in some of these lesions. I would say, as far as the bacteriology there is concerned, that we had better keep our fingers crossed since we don't know what life is and I don't believe our present techniques are adequate to determine that as far as the tubercle bacillus is concerned. Therefore, in these necrotic lesions, if they do soften up and slough out, the bacteria may find it possible to carry on their nefarious work.

Dr. Tucker: Thank you. Nick, would you give us your perspective on this question? Perhaps you might put it in general terms in much the same way as you discussed it at lunch this noon.

Dr. D'Esopo: I think that all open lesions should be regarded as harboring viable tubercle bacilli and I think we shouldn't stand before X-rays and try to guess whether these round shadows we see are cavities or blebs or cleaned-out cavities or healed cavities, and so forth. We don't know, and at the present time there is no way of knowing. We must assume that these are tuberculous cavities and that they harbor viable tubercle bacilli and that the patient has a good chance of relapse. We should do something about these open lesions surgically. As for the closed lesions, the question is of viability in terms of duration. In the Sunmount material, we found that in almost no instance in the patients who were resected after eight months of chemotherapy could we find viable tubercle bacilli. We have come to regard that as certainly the minimum duration of chemotherapy and then we have added four months to make it safe. In a way, our duration protocol may answer this question because the target point has three parts to it and two of them I think refer to the openness of lesions. In one case you see a cavity, and in the other case the culture is positive. The third criterion, stability, I'm not so sure about. But at any rate I think at the target point the lesions are closed as far as we can judge clinically. So what we are really doing is treating some patients six months after that time and some patients twelve months after that time, and we just don't know the answer.

Dr. Tucker: Well that implies to me—and see if I am expressing the opinion of the panel—that in the closed lesion, in which the cavity has been filled in or has never sloughed, the data available on viability of bacilli in resected tissue should not be taken into account in determining the duration of therapy. Do I have agreement on that?

Dr. D'Esopo: Except in terms of minimum duration.

Dr. Tucker: I think that's very important. Dr. Medlar, would you like to say something?

Dr. Medlar: Dr. Tucker, I'd like to add a modifying phrase to this viability—viability with present techniques.[1]

[1] *See* Hobby *et al.*, Am. Rev. Tubere., 1954 , *70*, 191.—*The Editor*

Dr. Tucker: Thank you. We all know to what you are referring. Dr. Jenkins, in a cavitary sputum-"positive" case, how long is one justified in continuing medical management of any kind with drugs, rest, collapse, and so forth, in an effort to attain the target point?

Dr. Jenkins: In my opinion and from our data, I would set this point at a year. I feel that way because we have seen, between the six- and twelve-month observation periods, cumulative increases in cavity closure amounting to about 20 per cent and cumulative increases in sputum "conversion" amounting to about 25 per cent.

Dr. Tucker: That's very interesting.—Dr. Mitchell?

Dr. Mitchell: I think our case material must differ considerably from Dr. Jenkins'. I know it does because we have only about 20 per cent of far advanced cases. In our experience with those with cavity at the outset, most of the cavities which are going to close during a subsequent period of observation in a total of twelve months or more will have closed within six months. And the same is true of sputum conversion. So that, in our group of cases, predominately moderately advanced, we feel that six months is the time at which to determine to use some other adjunct to aid the chemotherapeutic regimen.

Dr. Tucker: Nick?

Dr. D'Esopo: Yes, I agree with Roger. At about six months we feel that we must do something. Now we are just a little bit prejudiced about isoniazid, but we don't know that. We tend to delay a little more—it is mostly a question of feel rather than knowledge.

Dr. Tucker: I think the answer to this question clearly is or would be based upon studies of rates of cavity closure without surgical intervention. And, obviously, by holding off, one will accumulate this data. One does so at a certain risk because there is a widespread impression that, if we delay the surgical intervention directed toward a persistent cavity which is not closed with a shorter period of therapy, there are certain additional complications. Dr. Paulson, how do you feel about it?

Dr. Paulson: I feel that surgical intervention should be seriously considered in such a case after six to eight months of therapy, and I feel that must be considered because of the emergence of resistance after that time. No surgeon likes to be confronted with a patient with a high degree of resistance.

Dr. Tucker: It sounds as though Dan Jenkins were in the minority, but he may have something there. Nick, I think this question has really been referred to already. What is the prognostic significance of a persistently "negative" sputum by culture in a patient with an open cavity who is on original antimicrobial treatment?

Dr. D'Esopo: From a practical point of view, we must assume that the sputum will become "positive" again. I think Jim Raleigh, summarizing the Sunmount data yesterday, said that 50 per cent or so of those patients with open cavities and negative cultures finally relapsed. In some of these, at least, these cultures were "negative" for six months. In other words, the cavities were open and the cultures were negative for six months, chemotherapy was stopped, and the patient relapsed.

Dr. Tucker: So you would regard the X-ray evidence as more important than the bacteriologic?

Dr. D'Esopo: That's true, and I might say this, that this practice of ours of getting excited about open cavities is related to the paucity of drugs we have. Ideally, we should wait and have these patients relapse bacteriologically. If we had enough drugs, we would simply re-treat them and apply surgical measures. So this is a phase—I hope it's a phase—we are going through of doing everything at once because we just don't have enough drugs to get a second crack at the patient.

Dr. Tucker: You've just referred to the question of re-treatment. Actually, at your panel day before yesterday, this was discussed at some length and this is such a big and

complex problem, particularly as we employ a greater number of antimicrobial agents, that we could spend a great deal of time on it. To simplify this, I'd like to propose the following approach and then see if the members of the panel care to amplify it. In re-treatment with antimicrobial therapy, a general guide might be somewhat as follows:

With relatively little previous drug treatment with a particular drug, it is safe to use that same drug on re-treatment. With a moderate amount (deliberately not defined) of previous use of a specific drug, there has probably been some loss of susceptibility and it is probably best to use another drug instead to which the patient's bacilli are more likely to be susceptible. With a great deal of previous administration of a specific drug, it is practically always advisable not to use that drug again. Further, it is desirable to use the two drugs at least to which the patient is most likely to be susceptible on the basis of either tests or probabilities from previous experience, and it is highly important to guide one's therapy both at the outset and during the course of re-treatment with *in vitro* tests of drug-susceptibility. Now, would there be fairly general agreement with such a policy? Dr. Mitchell is good enough to nod his head. Dr. Hall?

Dr. Hall: I think I would agree with that. However, I think that, if a patient had had only a moderate amount of one drug, it would be safe to use that drug awaiting susceptibility tests and to change to a third drug only if resistance were proved.

Dr. Tucker: Thank you. Dan?

Dr. Jenkins: No additional comment.

Dr. Tucker: Don? Nick?

Dr. D'Esopo: I don't want to be a nuisance, but don't you think you have to define *little*, *moderately*, and *great deal*?

Dr. Tucker: I don't want to this afternoon, Nick.

Dr. D'Esopo: I don't think the question is very useful if you don't do that.

Dr. Tucker: I'll accept that criticism. I think we covered it quite well at your panel the other day. Yes, Dr. Mitchell?

Dr. Mitchell: I'd like to add one comment. Although the majority of cases will follow Bill's outline, they will be surprising when you do sensitivity tests and, if you can wait until sensitivity tests are run, you sometimes will plan treatment more effectively than if you go right ahead.

Dr. Tucker: I think that's very pertinent. I would like to ask anyone who cares to answer what he regards the present status of pyrazinamide in the treatment of tuberculosis to be. Well, I'd like to take a crack myself at answering since you are so modest. I will merely quote Dr. Spencer Schwartz, of Oteen, North Carolina, who gave a very interesting report on their studies to a small group of us the other day which was generally to the effect that pyrazinamide alone or in combination with streptomycin or PAS has a limited effectiveness; that it is impossible to measure by techniques presently available the emergence of pyrazinamide resistance (and it is only by judging the clinical course that one is able to estimate this, but it appears to occur beginning about the fourth week and to become quite rapid in six to eight weeks). When pyrazinamide and isoniazid are combined, when the patient has not had prior isoniazid therapy, there is a much longer period of effectiveness; and, if there is re-treatment with isoniazid in the face of previous isoniazid therapy, this also does not hold. I hope I have quoted Dr. Schwartz correctly on this. We obviously await further studies on pyrazinamide, but it may well be that, in the future, pyrazinamide will be an additional very important drug. It also has a place in short-term therapy in helping a patient to get past a critical point. Does anyone want to add anything to that?

It is obvious that the facts about ancillary measures such as collapse therapy do need

to be re-evaluated in the light of modern chemotherapy. We cannot possibly go into all of them, especially as we spent a session on this in Los Angeles last year, and this discussion was published in the December issue of THE AMERICAN REVIEW OF TUBERCULOSIS. A few questions I believe on this question are pertinent. For any patient with extensive disease, who is a respiratory cripple or who has a complicating illness, or for any other reason is not a good candidate for collapse or resection, what type of drug regimen should be employed? Now this question is in several parts. Initially, Dr. Mitchell?

Dr. Mitchell: If a patient cannot take either collapse or resection? And he's never taken chemotherapy before? I think I would give him streptomycin–PAS.

Dr. Tucker: After resistance has developed to one of those drugs, then what?

Dr. Mitchell: If resistance develops to one of those drugs, I think I would give him all three.

Dr. Tucker: Continue the one to which he is resistant and two more? Referring to streptomycin, isoniazid, and PAS?

Dr. Mitchell: The reason being that, as soon as resistance develops to one, it is very likely that resistance to the other is in the process of developing, and it seems to me it makes more sense to give all three than just two.

Dr. Tucker: And the reason for continuing the one is that there may still be some usefulness in it. After resistance has developed to all three of those, then what?

Dr. Mitchell: I'll pass—I would certainly continue isoniazid in spite of the development of isoniazid resistance. I want to make that very clear.

Dr. Tucker: Dr. Jenkins?

Dr. Jenkins: Which one of these do you want me to answer, Bill?

Dr. Tucker: After resistance has developed to all three of these drugs, how would you treat such a case?

Dr. Jenkins: This is a little hard on the patient and I don't know the answer to it, but we have pursued a policy of keeping all three in and then adding one of the other lesser drugs, such as viomycin or pyrazinamide.

Dr. Tucker: And Dan, after resistance to all known drugs has developed, what are you going to do?

Dr. Jenkins: We would certainly continue isoniazid and, as far as my experience allows me to say, I think we would probably continue other drugs that we had used as well.

Dr. Tucker: This is carrying things to the ultimate, but we do have those problems occasionally. We have some in Minneapolis.

Now to turn to pneumoperitoneum. Dr. Mitchell, does pneumoperitoneum have value with prolonged antimicrobial therapy in original treatment cases in any type of case of pulmonary tuberculosis?

Dr. Mitchell: No.

Dr. Tucker: Whoops!

Dr. Mitchell: Oh, I'm sorry, I thought you meant all . . . . Yes.

Dr. Tucker: And, if so, in what type?

Dr. Mitchell: Well, admittedly a retrospective study; Dr. Geoffrey Brinkman went over cases with Jim Raleigh at Sunmount and at Trudeau and selected 66 patients with cavity and "positive" sputum on twice-weekly streptomycin and daily PAS, some of whom had had pneumoperitoneum and some who did not. He found, to sum it up, that pneumoperitoneum caused a faster and a higher incidence of cavity closure in those patients with cavities of two or more centimeters.

Dr. Tucker: If one does use pneumoperitoneum, when should it be started in relation to the start of antimicrobial therapy?

Dr. Mitchell. I also have a rather strong bias on that score—I suppose because we had a few patients started in the fourth, fifth, or sixth month of treatment who have not responded too well. I think it should be started in the first month.

Dr. Tucker. And how long would you keep up the pneumoperitoneum?

Dr. Mitchell. Until a month or two after resection or until a year after the attainment of target point, roughly speaking.

Dr. Tucker. Thank you. Dr. Jenkins?

Dr. Jenkins: Judging from results in our study of advanced patients, I would agree with Dr. Mitchell. Our retrospective analyses would seem to indicate that patients given pneumoperitoneum early have done better.

Dr. Tucker: We know also that there is a study going on in the Veterans Administration in which there is random selection between pneumoperitoneum and no pneumoperitoneum under certain conditions which should answer this question more precisely. Do you have any additional comments, Dr. D'Esopo?

Dr. D'Esopo: I think the impressions of good observers are always useful, and I refer to Dan and Roger. They are good observers and what they have are impressions. But I don't think we have any facts. That's what I hope this study you referred to will do. It seems to me that one should want to have chemotherapy as definitive therapy; that should be our goal. We should want to get patients better without rest or without pneumothorax, pneumoperitoneum, without anything but chemotherapy. Now we can't do that, obviously. The only way we can learn the potentialities of chemotherapy is to withhold these other things, and I think that Roger and Dan are really not learning as much as they might concerning the potentialities of pneumoperitoneum. It is true that in this process of learning some patients might suffer, but that's the way it is any time you learn anything in medicine. That's why we withhold pneumoperitoneum.

Dr. Tucker: Dr. Medlar, do you know of anything from the pathologic standpoint which might bear upon the advisability or inadvisability of employing pneumoperitoneum therapy along with prolonged antimicrobial therapy?

Dr. Medlar. Mr Chairman, you're way over my head.

Dr. Tucker: Well I'm going to drop pneumoperitoneum at this controversial point and ask Dr. Paulson this next question. Can you describe an instance in which you would use pneumothorax in which resection would not be as appropriate or even more appropriate as a form of therapy?

Dr. Paulson: Repeat that again, will you?

Dr. Tucker: Can you describe an instance in which you would use pneumothorax in which resection would not be as appropriate or even more appropriate as a form of therapy?

Dr. Paulson: I'll say no, although I'm still not clear on that.

Dr. Tucker: We'll let him read it and see if such a long sentence parses better that way. Let's put it this way. Here's a given lesion for which one might consider pneumothorax. Faced with such a situation, can you ever think of a situation where you would select pneumothorax deliberately instead of resection?

Dr. Paulson: No.

Dr. Tucker: Thank you. That's my only question on pneumothorax. No, it isn't either, I beg your pardon. I'll ask Dr. Paulson another one about pneumothorax—not that I imply that he is an advocate of pneumothorax. Are the risks of resection greater than those of pneumothorax used to treat the same type of lesion—with prolonged antimicrobial therapy, of course?

Dr. Paulson: I would say not.

Dr. Tucker: Thank you very much. And, Dr. Paulson, another question. Can you think of a single instance where you would use a phrenic nerve operation today in the treatment of pulmonary tuberculosis?

Dr. Paulson: No.

Dr. Tucker: Is there any comment on this? Dr. D'Esopo?

Dr. D'Esopo: No.

Dr. Tucker. Dr. Mitchell? Dr. Jenkins?

Dr. Jenkins: I'd like to ask Dr. Paulson what is the minimal amount of function that he would like to have in the contralateral lung when he does a resection of any type on the opposite lung?

Dr. Paulson: You mean in actual measurement?

Dr. Jenkins: I was thinking in terms of resection versus pneumothorax. What is the minimum amount of function you would like to have in the opposite lung before doing resection?

Dr. Paulson: I find it difficult to state. I would like to have adequate pulmonary function, and I think that should be taken into consideration in all types of resections. After preliminary investigation, it should be felt that the patient will have adequate pulmonary function to carry him on after the resection. I assume that was the consideration in answering the question

Dr. Tucker: Do you want to help him out and get a clearer answer, Dan?

Dr. Jenkins: I think my only comment is that I rather felt that one could get away with pneumothorax where perhaps one could not get away with resection, the contralateral lung having minimal function.

Dr. Tucker: Don? Does that help any?

Dr. Paulson: I still don't think that that was quite the question. The question seemed a little ambiguous at the time.

Dr. Tucker: Go ahead now.

Dr. Paulson: I assumed you had a choice in the question from a functional standpoint in regard to either pneumothorax or resection.

Dr. Tucker: Roger, do you have a comment?

Dr. Mitchell: I can think of three situations where a pneumothorax would be preferred to a resection: where the opposite lung is damaged, where the lesions are widely scattered, and where good thoracic surgical service is not available.

Dr. Tucker: You would concede that collectively these do not constitute a large place for pneumothorax?

Dr. Mitchell: Yes. Do you want me to answer the question about phrenic?

Dr. Tucker: Sure.

Dr. Mitchell: I don't think there is a place for phrenics. We don't know about the question of pneumothorax complications. Everyone who has reported on pneumothorax, using it during prolonged combined chemotherapy, reports few cases of empyema. I have not yet heard of a series in which a few cases have not been reported. No one has yet told us the outcome as far as function is concerned, that is, unexpandable lung. I do agree with the opinion expressed that the risks of pneumothorax today still seem as great as, or greater than, those of resection.

Dr. Tucker: Dr. D'Esopo, is there any longer any justification for a conventional thoracoplasty in the light of present results with plombage thoracoplasty?

Dr. D'Esopo: I don't know. We at West Haven use plombage, but I really can't answer that question. I don't know enough about it.

Dr. Tucker: I'm still going to stay away from Dr. Paulson for a moment. Dr. Jenkins?

Dr. Jenkins: I can't answer this question as stated, Bill, because I don't have sufficient experience with it.

Dr. Tucker: Another pass. Dr. Mitchell?

Dr. Mitchell: This is a dirty trick, Bill. I wrote this question.

Dr. Tucker: I knew you did.

Dr. Mitchell: I expected you to ask someone else. I should answer the question in the way Dan has answered it in that I have not had enough personal experience. But, judging from the experience that I have heard quoted, I would say that plombage thoracoplasty might very easily take the place of conventional thoracoplasty, that is, when the periosteum is displaced medially and a new set of ribs is grown with a permanent plombage.

Dr. Tucker: All right. Don, I'll ask you the question. Is there any justification for conventional thoracoplasty in the light of results with plombage thoracoplasty?

Dr. Paulson: Yes, I feel there is definite justification. I am aware of the advantages of extrafascial Lucite plombage in so far as preservation of function is concerned, and the results in the hands of others have been reported as very satisfactory. We have used it, not extensively. We are pretty good thoracoplastic surgeons, and we tend to rely more on the conventional thoracoplasty. By that, I don't mean a total thoracoplasty; I mean a tailored thoracoplasty.

Dr. Tucker: Would you give us briefly your main indications for a conventional thoracoplasty?

Dr. Paulson: We believe that thoracoplasty is an operation which competes with pneumonectomy. We will think of a primary thoracoplasty in preference to a pneumonectomy in many, many instances. This does not mean that we do not do pneumonectomy. But in the case of involvement of more than one lobe, the very common distribution being an upper lobe with the superior segment of the lower lobe, with destruction and cavitation, we prefer thoracoplasty because, if a resection is done, more than one lobe or portions of that lobe must be subjected to resection. The morbidity increases as a result, and a concomitant or follow-up thoracoplasty will have to be done in many of those cases to reduce the size of the space. So, in those, we will tend to do a primary thoracoplasty. Also, in the cases of destroyed lung, we feel that a primary thoracoplasty is a safer procedure, with a lower morbidity and mortality rate. The complications admittedly being less at the time, it is planned that a resection may be necessary at a later date. But we feel it can be done far safer under the thoracoplasty. "Positive" sputum, cavity, resistant tubercle bacilli are other factors favoring thoracoplasty.

Dr. Tucker: Would you care to say, Don, to what extent the opinion you have just stated is shared by other thoracic surgeons?

Dr. Paulson: Well, sir, I think that it is shared by some others in our area. I can't say exactly how far that goes.

Dr. Tucker: I do gather that there is quite a rebirth of interest in thoracoplasty, at least in some portions of the country, and I suspect we shall be hearing more about this. Now, to come to a few questions on resection. Dr. Medlar, would you care to say what you think might be the indications for a clinician to keep in mind for the resection of a closed necrotic residual tuberculous lesion?

Dr. Medlar: There's only one thing I think the clinician should keep in mind after he turns the case over to the surgeon and that is that the surgeon, in taking out the lesion, may milk out some necrotic material from some of the necrotic lesions; therefore, he would have to be very sure that the chemotherapy is going to be continued for the proper length of time after surgery.

Dr. Tucker: Dr. Hall, do you think it is desirable to resect most residual necrotic lesions or, if you feel that any should not be resected, which ones?

Dr. Hall: I think it is true that most of them should be resected. I think it is simply impossible to resect them all. It is obvious that they are not even all palpable by the surgeon in those cases which are operated; that many are left behind. I think that the judgment whether they should be resected should depend upon the risk. In the poor risk patient, it is obvious that sometimes we can't remove when we would like to. Also, I think that some attention has to be paid to the duration of the disease and its probable reversibility. I think that, if one has a patient with acute disease which is rather widely disseminated and which after prolonged chemotherapy leaves behind only widely scattered and very small residuals, particularly if some of them are calcified, I would elect to leave them.

Dr. Tucker: Dr. Mitchell, what would be your selection of resection for target-point lesions?

Dr. Mitchell: Bill, the evidence available to date indicates that resection does not improve the results of tuberculosis treatment when the target point is reached as a result of prolonged combined chemotherapy.

Dr. Tucker: And for a period afterward, as far as data are available?

Dr. Mitchell: The data available so far amount to 182 cases combining the experience at Trudeau and Sunmount, and in those two groups there has been no difference in the behavior in the period of follow-up of one to four years after discontinuation of chemotherapy.

Dr. Tucker: So you would be in favor of resecting none of them?

Dr. Mitchell: Well, just to take a point of view, I'm not at all sure it will be the final answer, but that is the point of view we have taken.

Dr. Tucker: So you are resecting very few target-point lesions or none? Is that right?

Dr. Mitchell: Once in a while we feel inclined to resect because of the patient's personality and his unwillingness or inability to cooperate with the treatment regimen after leaving the sanatorium.

Dr. Tucker: Dr. Jenkins, what are your indications for resection of the target-point lesion?

Dr. Jenkins: We have resected very few of them. I might say, though, that those are the ones that our surgeons wish to resect. Our relapse rate in about 110 target-point lesions in which resection did not assume any place in arriving at the target point, followed for four months to three years, with an average follow-up of about a year and a half, is around 6 per cent. For whatever that figure is worth, I think it is roughly comparable to the other data that we have heard at this meeting.

Dr. Tucker: Dr. Paulson?

Dr. Paulson: I don't think they should be removed routinely. I think, though, that the age of the patient, the distribution of the nodules that remain must be taken into consideration, and how close the patient can be followed after his discharge from sanatorium care. In a young person in our area who is going into an area where follow-up will not be very close or perhaps adequate and, if the nodules can be removed by segmental resection, we will do it. We are not sure that those treated only by antimicrobial therapy are going to stand up over a long period of time, and we don't know if we are doing the right thing or not; but we have carried out such policy.

Dr. Tucker: Dr. D'Esopo?

Dr. D'Esopo: I'm sure that no one knows whether these things should be resected or not, but one must act while attaining this knowledge. One of the most difficult things in tuberculosis today, I think, is to remain without convictions, without strong opinions.

There is so much data—everyone is working on these problems—that it is extremely difficult to simply be ignorant. I think one must discipline oneself. We really don't know whether to take these things out or not, and at West Haven we do remove residual necrotic lesions. We know what they look like from Dr. Medlar's studies. We know they're necrotic, and in most of them there is a communicating bronchus; there are the morphologic prerequisites for relapse. We're not sure of the viability. It is my personal opinion that these organisms are dead, but I don't know that. And a good guess is not a fact. We can only get the answer to this problem by follow-up of the unresected and the resected, and there hasn't been enough time. As Jim Raleigh showed yesterday, the unresected do quite as well as the resected in comparable cases, but not enough time has elapsed for the observation of these two groups. I might just add that I believe the resection of these closed lesions is an operation of election. We don't urge the operation on patients; we simply say that we think that in the end your prognosis will be better if this is out, but we don't urge it.

Dr. Tucker: Nick, how much additional observation do you think we'll need before we can get some firmer basis for answering this question?

Dr. D'Esopo: Oh, I don't know. We have followed them now for four years; a good round number, I should say, would be ten years.

Dr. Tucker: It is obvious that there is a disagreement—Yes, Dr. Medlar?

Dr. Medlar: I have to get into this argument. One point to uphold the surgeon: I'm sure that surgeons can feel lesions in the lung that are not over a millimeter or two in diameter. And I doubt very much if they miss much, if any, in their palpation of the lung during the surgical procedure, that is, if they are looking for it. Now, there is one other thing about this comparison of the patients who have not had resection with those who have had resection. The ones without resection are put down as failures of chemotherapy. Well, then let's put the ones that have had chemotherapy and resection down as a failure of chemotherapy and surgery. That is, it's a failure of chemotherapy whether it is resected or not, and I think that should be borne in mind.

Dr. Tucker: I think it is obvious that we don't have the answers to this and that there is a wide variation in practice, and I don't believe the panel can give a definitive answer to any greater extent. I think, however, there is more agreement on the surgical approach to the non-target point lesion, either when the sputum stays "positive" or when a cavity persists. Would the panel in general agree that there is an indication in such situations for either resection or a collapsing procedure such as thoracoplasty? Dr. Paulson?

Dr. Paulson: I would agree. Either "positive" or "negative."

Dr. Tucker: I think I saw a number of heads nodding. Does anyone care to take exception to it? I think you are in fair agreement with that, and in my opinion, this is one of the chief indications for resection today. And with that I am going to change in the last moments of the panel, and we will take a few minutes past 5:30, because we got started late, to get to the subject of bed rest. I'll start out by asking Dr. Mitchell what in his opinion has been the justification for the use of bed rest in the treatment of tuberculosis in the prechemotherapy era?

Dr. Mitchell: I think bed rest got started in various places in different ways. But, as time went on, the ideas about why bed rest was effective began to crystalize in people's minds: the reduction of metabolic activities which decreased the need for oxygen; the horizontal position which caused a rise in the diaphragm, reducing the volume of air in the lung, causing a mild bilateral collapse, and possibly properly circulating the apices following Dock's theory. Those are the arguments commonly put forth. There is another one, of course, general immunology, general care improving the person's ability to fight infection.

Dr. Tucker: Are you pretty much convinced, Roger, that, before antimicrobial therapy was available, bed rest assisted in getting better results generally.

Dr. Mitchell: There is no question of that.

Dr. Tucker: With that very brief reference to the ancient past, Dr. D'Esopo, in your opinion, with modern antimicrobial therapy, is bed rest less necessary today than formerly?

Dr. D'Esopo: I rather feel so, but again—it seems to me that throughout this whole meeting all I've been saying is "I don't know" and I don't know this either. But I rather feel that less rest is necessary. This to me is the important thing that we always knew, that patients got well without rest. We say that, as soon as we got X-rays, we saw patients with healed lesions who had never "cured." The real question is not whether patients get well with ambulatory chemotherapy, but what kind of patients. And you say what does the phrase "what kind of patients" mean? I think it implies a classification of patients in terms of their bacteriology, in terms of their pathology as interpreted by X-ray, and in terms of their chemotherapy. On the other hand, it implies a classification of patients in social terms, economic terms, psychological terms, and so forth. That's what we really need to know. Otherwise, I think that we cannot communicate with each other. If one describes a hundred or two hundred cases treated with ambulatory chemotherapy either before hospitalization or afterward, the first questions that should come to one's mind are, who are you talking about; what kind of cases are they? And that I think is the thing we have to answer in the future.

Dr. Tucker: I really thought that I had phrased that question in such a way that you couldn't help answering yes. The question was, "With modern chemotherapy, in your opinion, is bed rest less necessary than formerly?" And I thought I almost got a "yes" out of you.

Dr. D'Esopo: I said I think so, but I really don't know.

Dr. Tucker: The rest of your remarks were extremely pertinent.

Dr. D'Esopo: That's what I was trying to say in commenting on the other three papers. I didn't get the chance and I thought I would do it here.

Dr. Tucker: As a matter of fact, I have one brief comment to make in reference to the three papers. I think that there was an unparalleled opportunity, particularly in the two large cities of New York and Chicago, to have embarked upon a study which would have given answers to a greater extent than are presently available, and that would have been by assigning patients in the categories in either of those two studies on the random-selection basis to ambulatory chemotherapy and hospitalized conditions. This bears directly on what Dr. D'Esopo has referred to, namely, the comparability of material, and it is only by assignment by random selection that I think we can be at all confident where measurements of difference are likely to be small. Dr. Mitchell, do you think that all patients should be hospitalized at the start of antimicrobial therapy when there are adequate facilities?

Dr. Mitchell: Yes, I do, and my reasons, if I may give them, are that it is difficult to get patients to follow out the instructions about their chemotherapy and continue it long after they have been feeling well: to take PAS when it makes them sick to their stomachs, to do all the things to follow themselves up closely unless they have been brought into realization of the nature of tuberculosis. The best way to learn that is in contact with with other patients, with nurses, doctors, and other staff members who can teach these things to them.

Dr. Tucker: I'll ask you another question which is really the reverse of this. What may be the justification for the use of bed rest in a patient who is asymptomatic with a minimal lesion but who is a mature cooperative adult?

Dr. Mitchell: I would be willing under very special circumstances and with select individuals to give that person a discharge after two months, provided that he had got by that period without drug hypersensitivity or intolerance and showed the factors mentioned in the question.

Dr. Tucker: But for the other reasons you have stated you would still prefer to start therapy in the hospital.

Dr. Mitchell: I meant to say this patient should start in the hospital.

Dr. Tucker: I think that Dr. Lichtenstein's report very clearly showed that the problem of post-sanatorium chemotherapy is rather different from pre-sanatorium chemotherapy, and I think there were some data in Dr. Robins' report which suggested somewhat the same. Dr. D'Esopo, would you care to say whether you think it is ever justifiable to treat a cooperative patient with a minimal lesion entirely on ambulatory chemotherapy?

Dr. D'Esopo: I think, in general, I agree with Roger that they should be started in the hospital because of the observations that are possible there; but in exceptional cases I should think it would be justifiable to do everything at home.

Dr. Tucker: I think we are all aware of the fact that there undoubtedly are going to be changes and that we should move rather gradually and test out our steps as we go along. If I understood Dr. Dressler's report, however, with respect to one important aspect of this problem, namely, decreasing the amount of rest in the hospital, he was deliberately testing out a decrease in the amount of rest. So I would like to ask the panelists what degree of rest in the hospital they think may be necessary with modern treatment with drugs in a patient with a good prognosis? Dr. Jenkins?

Dr. Jenkins: My answer will merely be an opinion on that. I feel that the patient should be initiated on therapy with confining bed rest. The allowance of bathroom privileges would meet that criterion in my opinion. Subsequent to the disappearance of symptoms, such as fever, cough, and sputum, I think that the rest program could be liberalized. Beyond that I can say nothing specific.

Dr. Tucker: Dr. D'Esopo?

Dr. D'Esopo: I think some patients can be started on a fully ambulatory basis the first day they come in the hospital; again, as part of a study.

Dr. Tucker: I think we all agree that, after the initial period of restriction of activity—and I'm deliberately vague about that—if the course of the disease in a particular patient is favorable, we can increasingly diminish the amount of restriction of activity, to put it very negatively. I think we had best avoid the details in this panel discussion. But I can ask this general question. What may be the dangers of ambulatory chemotherapy at the present state of our knowledge? Nick?

Dr. D'Esopo: The chief danger is the one I referred to earlier, that we may not all be talking about the same case. At New Haven, we have an extremely highly selected group of patients on post-discharge chemotherapy. And I suppose we might loosely call that an ambulatory program, but it has no resemblance whatsoever to some of those programs where patients are treated before hospitalization because there are no beds. That to me is the chief danger, that is, one of communications and semantics. The other danger is this: I think patients should not be discharged from the hospital unless their cultures are "negative" and unless they are expected to stay "negative." In other words, I wouldn't discharge a patient from the hospital on drugs if he had an open cavity, no matter how long his cultures were "negative." I would like to be able to say that these cultures are not only "negative" but they will stay that way.

Dr. Tucker: Do you think, Nick, that such patients, the highly selected group to which you have referred, may return promptly to work with safety?

Dr. D'Esopo: We are doing that; again, as a part of the study. I think we must try to

get an answer as rapidly as possible and we are really straining the idea. We are allowing these patients to go back as soon as they are discharged if they can get jobs, and to go back in most cases to their former occupations. If this is really going to work out—or, rather, if it is going to fail—I think we had better know about it as soon as possible so that the structure of tuberculosis treatment, all the things that have been built up in the last fifty years, won't be hurt. We should try to get a fast answer, and I think we can get this fast answer by straining the ambulation.

Dr. Tucker: Do you place any limitations on the type of work—any job that they want to take?

Dr. D'Esopo: In extreme cases, I think we still adhere to tradition. If somebody is an extremely hard worker, we don't feel very good about asking him to go back to that, but for the usual occupations we ask the patient to go back to what he was doing before. It really is very helpful because many of the patients have to buy their own drugs, and it is easier for them to get a job if they go back to what they were doing.

Dr. Tucker: Nick, still, in patients with a bad prognosis, with open cavity, "positive" sputum, should ambulatory treatment ever be advised?

Dr. D'Esopo: Oh, I think not, because the only justification for ambulatory treatment, I think, is the success of chemotherapy and, if you think that chemotherapy is going to fail or if you know it has failed, then I think we should not use it and we should depend on rest. I can't overemphasize the fact that we must know the kind of case in pathological terms.

Dr. Tucker: I think there probably are some cases in which you would advise outpatient chemotherapy on an ambulatory basis, and I think they were referred to in Dr. Lichtenstein's paper as "maximum hospital benefit." This is perhaps not the most accurate way to designate that group.

Dr. D'Esopo: Oh, yes, we have a few patients whose home situation is good. We first get in touch with the health people and say this person is seventy years old and he lives with his wife and they have a good home and so forth, and he's "positive" and we'd like him to go home. Those should be very few cases.

Dr. Tucker: Just one last question, and I want to read it carefully and to see whether I do have general agreement from the panel. May we, on the basis of information presently available, look forward with confidence to a further reduction in the amount of bed rest and hospitalization which will be necessary with better drug therapy and other ancillary measures probably available? Would you agree that we may look forward to that, Nick?

Dr. D'Esopo: Yes, I'm rather convinced of it, but again I don't know it.

Dr. Tucker: Dr. Paulson? Dr. Paulson agrees. Dr. Jenkins?

Dr. Jenkins: I agree, with caution.

Dr. Tucker: And Dr. Hall?

Dr. Hall: I certainly believe that's the case.

Dr. Tucker: And Dr. Medlar.

Dr. Medlar: I'm a pathologist. I believe I have no right to vote on matters pertaining to the clinical management of tuberculous patients.

Dr. Tucker: And, with that, we close this final session of the American Trudeau Society.

The Treatment of Tuberculous Lymphadenitis

A Report by the Committee on Therapy

The treatment of tuberculous lymphadenitis has received little special study since the introduction of specific antimicrobial therapy, as compared with the treatment of pulmonary tuberculosis and of other forms of extrapulmonary tuberculosis. This comparative neglect probably results from the relative infrequence of tuberculosis of the lymph nodes as an independent or presenting manifestation of tuberculosis; and from the widespread impression that, when it does so present itself, especially as an apparently localized affection of superficial nodes, it is less serious than most other forms of tuberculosis in the human.

As a consequence, the recent literature concerning the treatment of tuberculous lymphadenitis is sparse, especially with regard to the results of antimicrobial therapy. Not only is there no consensus regarding the optimum treatment, but there is scarcely any formulated opinion except that of surgeons and radiologists, who are concerned primarily with the local aspects of treatment by excision or by roentgen irradiation. A recent analysis, however, of all of the patients with tuberculosis of the superficial lymph nodes discharged from the Toronto Hospital for Tuberculosis in the twenty-year period 1932–1952[1] indicates that this is uncommonly a localized form of tuberculosis and that it is no longer predominantly a disease of childhood, which it was in the first two decades of the century, when the excisional treatment of cervical lymph node tuberculosis was perfected by Dowd[2]. The principle of the complete removal of all involved nodes and cold abscesses which was established by Dowd has been followed by more recent advocates of the surgical excision of superficial lymph node tuberculosis[3-5].

There appears to be no doubt that tuberculosis of the superficial lymph nodes can be effectively treated by excision so far as the local result is concerned. There is also evidence that roentgen irradiation is often locally effective[6, 7] and that, with the lower dosages recently employed, the hazards of such therapy are not great. Reports of late follow-up results of either of these forms of treatment are limited, and there is little recognition of the possibility that superficial lymph node tuberculosis has become more commonly a manifestation of generalized tuberculosis than no doubt it was earlier. Yet, in Gale's recent series[1], previously cited, 88 per cent of the patients had associated tuberculosis in other parts of the body, most commonly in the lungs and in the bones and joints.

Both the surgical and the irradiation treatments are concerned principally with cervical lymph node tuberculosis and are predicated on the conception that tuberculosis of these lymph nodes is usually a localized form of tuberculosis, of which the portal of entry is the oro-pharynx. Tuberculosis of the tonsils, either from primary or reinfection, is held in this view to be commonly associated with or, indeed, responsible for the cervical lymph node tuberculosis. Pathologic and bacteriologic evidence that this is now frequently the case is lacking. On the contrary, there is much to suggest that cervical, no less than axillary, intrathoracic, or abdominal lymph node tuberculosis is most often a manifestation of generalized tuberculous infection.

[1] Gale, G. L.: Canad. M.A.J., 1953, *69*, 303.
[2] Dowd, C. N.: J.A.M.A., 1916, *67*, 499.
[3] Bailey, H.: Lancet, 1948, *1*, 313.
[4] Lester, C. W.: Surg., Gynec. & Obst., 1948, *87*, 719.
[5] Lester, C. W.: Am. Rev. Tuberc., 1951, *64*, 691.
[6] Lampe, I., *et al.*: Am. J. M. Sc., 1949, *217*, 632.
[7] Aceto, J. N., *et al.* Am. Rev.: Tuberc., 1953, *68*, 157.

Regardless of whether or not cervical lymph node tuberculosis is often associated with tuberculosis of the tonsil, there is little reason to think that it is frequently caused by infection with tubercle bacilli of bovine origin. Park and Krumwiede,[5] who typed the infection in 54 cases of cervical lymph node tuberculosis operated upon by Dowd, reported that only one-third were infected with the bovine type. Even this early (1910) infection with tubercle bacilli of human origin predominated except in the age group of less than five years. The subsequent virtual elimination of tuberculosis from dairy herds and the widespread pasteurization of milk have probably further diminished the proportion of infections due to bacilli of bovine origin, at least in the United States and Canada.

The available literature concerning the effect of antimicrobial therapy indicates merely: (*1*) that tuberculous lymphadenitis does tend to regress under such therapy, although often very slowly; and (*2*) that short-term, i.e., up to 120 days, therapy is frequently followed by local relapse or the development of active foci elsewhere. The published reports relate almost exclusively to streptomycin or streptomycin–PAS. Very few reports are available regarding long-term therapy with these agents, and even less has been published regarding isoniazid therapy in this form of tuberculosis.

In view of the paucity of information regarding presently available forms of antimicrobial therapy, not only with respect to results but even with respect to what is the most usual current practice, the Committee attempted to collect the experience of its own members and of others. Although the Veterans Administration, in its cooperative studies on the chemotherapy of tuberculosis, discontinued several years ago any central collection of data relating to lymphadenitis, the practice and experience of individual hospitals in the Veterans Administration were polled by the Committee. The information thus obtained added greatly to the volume of clinical material which could be considered.

As had been anticipated, the variability in treatment was so great that no statistical analysis of results could be attempted. The practice varied from the one extreme of surgical excision or roentgen irradiation with no concomitant antimicrobial therapy to the other extreme of long-term antimicrobial or simple rest treatment with no local treatment, except in exceptional circumstances, such as unusually large lymph nodes or persistence of sinuses. In so far as the effect on the lymph nodes themselves is concerned, those hospitals which employed long-term chemotherapy were satisfied with the results and usually reported a favorable effect during the maintenance of therapy. No significant data were yet accumulated relative to the incidence of post-treatment relapse. The longer excision of superficial lymph nodes was deferred, the less frequently was it still considered necessary. Sinuses, if present, usually healed, and cold abscesses regressed, although frequently with the aid of needle aspiration. No comparisons were possible between isoniazid and streptomycin–PAS since, when isoniazid was used, it was usually in combination with one or both of the other drugs. One observation of special interest, which was recorded several times, is that even lymph nodes which break down or first appear during antimicrobial therapy usually regress satisfactorily if the therapy is continued unchanged. This has been noted both under combined therapy and under isoniazid as single-drug therapy.

The consensus of the Committee, based on the literature and the unpublished experience which was reviewed, is that antimicrobial therapy is indicated in virtually all instances of active tuberculous lymphadenitis, as in other clinical forms of tuberculosis. The evidence indicates, however, that short-term therapy is not adequate and that long-term therapy is not yet established as independently capable of permanently arresting the disease in most instances. The extent to which such additional measures as excisional surgery,

[5] Park, W. H., and Krumwiede, C., Jr.: J. Med. Research, 1910, *23*, 205.

roentgen irradiation, and prolonged rest therapy are needed for the best results is as yet entirely undetermined. There is much to suggest that the management of lymph node tuberculosis simply as a local disease process without systemic treatment, although it may have had a sound rationale in the past, is rarely, if ever, justified at the present time. The importance of rest and sanatorium treatment should not be discounted, especially in early cases.

Obviously, there is need for more information, particularly of statistically significant numbers of patients treated in various fashions and followed for a considerable number of years. This form of tuberculosis is sufficiently important that its special problems merit particular study, and sufficiently prevalent that such study is practicable.

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NOTES

AMERICAN TRUDEAU SOCIETY FELLOWSHIPS

Resident Fellowships

The American Trudeau Society, medical section of the National Tuberculosis Association, provides a limited number of resident fellowships to promote the training of clinicians, medical teachers, and administrators in the field of tuberculosis and related pulmonary diseases. Awards are open to citizens of the United States for work within this country. Candidates should hold the degree of Doctor of Medicine and preference will be given to those not more than thirty years of age.

Awards are made for graduate study in internal medicine, with emphasis on diseases of the chest, in an approved hospital. The American Board of Internal Medicine will accept one such year as part of the prerequisite training for certification in internal medicine and in pulmonary diseases.

All awards are determined by individual circumstances and are paid directly to the Fellow on a monthly basis. Fellowships are granted for one year. Not more than two renewals will be considered. Fellowship applications must be received by February 1. Appointments may begin on any date at the convenience of the applicant, who may elect the hospital in which he wishes to study and the staff member who will supervise his work.

Trudeau Fellowships

A few fellowships at a higher level of training and award are offered to specially qualified candidates who have been assured of a continued teaching appointment upon completion of training. Trudeau Fellowships are awarded for one year but may be renewed up to a total period of four years.

Further particulars concerning Resident and Trudeau Fellowships may be obtained on request.

Address communications to the Director of Medical Education, American Trudeau Society, The Henry Phipps Institute, Seventh and Lombard Streets, Philadelphia 47, Pennsylvania.

ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY—1955

The American Trudeau Society, medical section of the National Tuberculosis Association, invites the submission of papers for the 1955 annual meeting to be held in Milwaukee, Wisconsin, May 23–27, 1955.

Abstracts

Abstracts of papers to be offered for presentation should be submitted in *duplicate* before January 1, 1955, with an absolute deadline of February 1, 1955, to Dr. David T. Carr, Chairman of the Medical Sessions Committee, 200 First Street, S.W., Rochester, Minnesota.

Abstracts should be limited to a 300-word summary including important data and conclusions. Charts and tables should be attached to aid in the selection of papers.

The names, titles, and complete addresses of the senior author and co-authors should be included in each abstract. The author who is to present the paper must be the first author listed in the abstract.

All abstracts and papers will be reviewed by the Medical Sessions Committee and, when necessary, by experts in special fields in order to select those containing the most interesting material. Final selection will be on a competitive basis.

Subjects for presentation may include all scientific aspects of tuberculosis and nontuberculous respiratory and cardiopulmonary disease as well as the results of investigative work bearing on these subjects.

Those wishing to present subjects of a confidential nature from the armed forces or other sources must clear the material through official channels *before* submitting the abstracts to the Chairman of the Medical Sessions Committee.

Papers

Subsequent to review of the abstracts by the Medical Sessions Committee, papers will be chosen on a competitive basis from among the abstracts for presentation at the annual meeting. Authors will be notified by March 1, 1955, if their papers are to be read.

A double-spaced typewritten manuscript must be prepared in *duplicate* to be presented to the moderator of the session at the time the speaker presents his paper at the annual meeting.

Papers presented at the annual meeting must be original contributions which have not previously been presented or published. All such papers should be considered as belonging exclusively to the National Tuberculosis Association and should not be submitted for publication elsewhere without the advance permission of the National Tuberculosis Association.

Abstracts of all papers given at the annual meeting will be published in the annual *Transactions* of the National Tuberculosis Association. All papers will be submitted to THE AMERICAN REVIEW OF TUBERCULOSIS, but publication in the REVIEW will be subject to the decision of the Editor.

The time allotted for presentation of papers at the annual meeting will be strictly limited by the committee in order to permit adequate discussion.

Participants in the program are reminded of the fact that travel expenses to the annual meeting are not paid by the National Tuberculosis Association.

SYMPOSIUM ON CHRONIC PULMONARY DISEASE

A Symposium on Chronic Pulmonary Disease, planned especially for general medical practitioners, will take place on Thursday, November 11, 1954, in the building of the Medical Society of the County of Queens, 112–25 Queens Boulevard, Forest Hills.

Sponsors are the Medical Society; the Queens County Chapter, Academy of General Practice; and the Queensboro Tuberculosis and Health Association.

The symposium will afford a comprehensive view of current concepts of the chronic pulmonary diseases, their diagnosis and treatment. It is approved by the American Academy of General Practice for five hours of formal credit.

The afternoon session begins at 2 p.m., and the evening session at 8 p.m.

For complete program information please call or write Miss Miriam Tauber, director, professional education, Queensboro Tuberculosis and Health Association, 159–29 90th Avenue, Jamaica, New York, JAmaica 6-2557.

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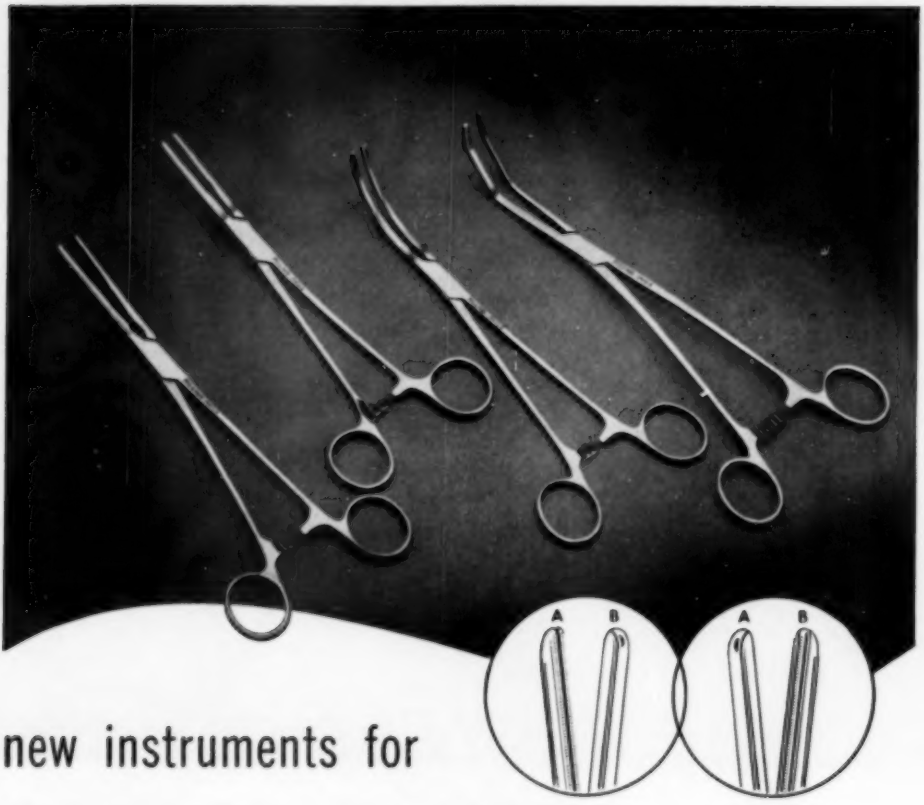

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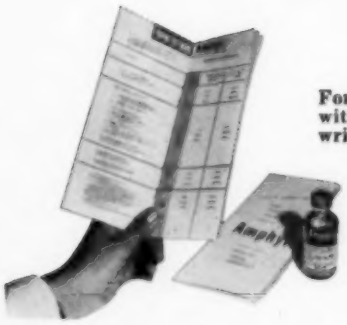
[1] *Smith, C. R.: Disinfectants for tuberculosis hygiene, Soap and Sanitary Chemicals 27:130 (Sept.) 1951; 27:143 (Oct.) 1951.*

[2] *Klarmann, E. G.; Wright, E. S. and Shternov, V. A.: Prolongation of the antibacterial potential of disinfected surfaces, Applied Microbiology 1:19 (Jan.) 1953.*



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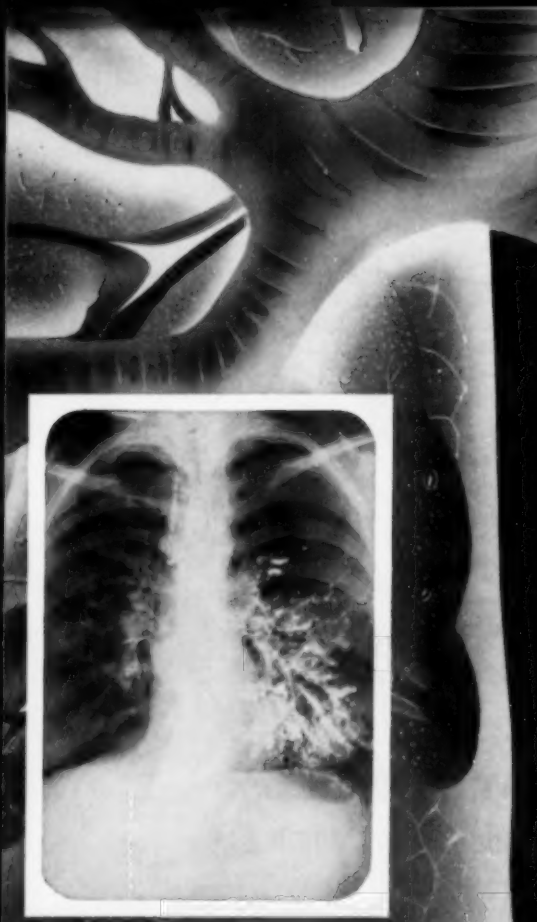

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Singer, J. J.: Differential Diagnosis of Chest Diseases, Philadelphia, Lea & Febiger, 1949, pp. 1953-4.

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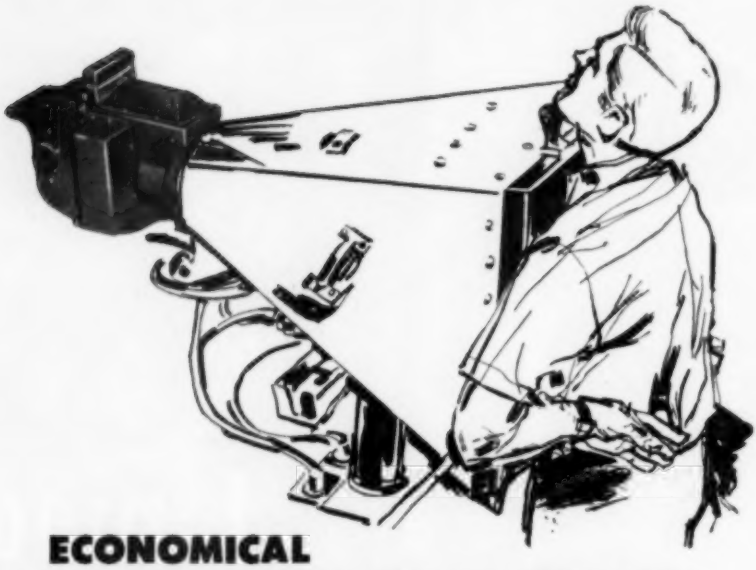

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Report of the Council of Tuberculosis Committees, American College of Chest Physicians, April, 1951:

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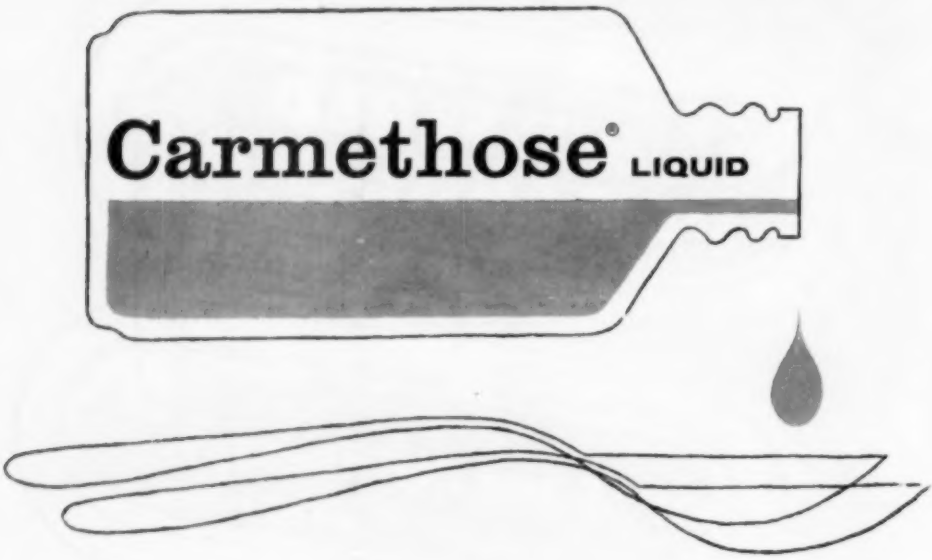

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